A RATIONAL MODEL FOR BOD-EXERTION IN STREAMS

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CERTIFICATE

This is to certify that the present work has been done under my supervision and the work has not been submitted elsewhere for a degree or a diploma.

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ABSTRACT

The inadequacy of the existing formulations describing the BOD exertion in streams is brought out clearly and attention is focused to the major significant role played by bacteria in the removal of BOD. A mathematical model incorporating the salient features of the BOD-reactions is suggested to describe the profiles of organic pollution in streams. It is founded on the Michaelis-Menten kinetics of enzyme-catalysed reactions. This rational formulation together with the Monod's equation for bacterial growth go to predict the pollution in streams to a fairly accurate degree. The solutions of these equations are worked out by digital computer techniques. An experimental investigation is presented for the verification of the model and the results indicate a striking agreement between the two. The application of the rational model is stressed for carrying out river-pollution abatement programmes based on more such realistic and rational approaches than on the existing crude formulations.

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CHAPTER I

INTRODUCTION

a. General:

For the maintenance of normally satisfactory conditions in a river, the oxygen economy is of paramount consideration. When pollutional nuisance of receiving waters is to be avoided, the DO and BOD, taken together, are generally relied upon to delineate the profile of pollution and natural purification on which engineering calculations of permissible loadings are based. Requisite evidence is rapidly accumulating on the inadequacy of the existing mathematical formulations to account for, in a more realistic manner, different variables that go to describe the nature and extent of pollution and the microbial population. In particular, the scope of the classical aspects of BOD-kinetics needs broadening to an extent that it will engulf a good deal of the already existing knowledge from the related domains like biochemistry, Chemical-kinetics etc. The obvious rational approach will be to lay stress on the formulations of mathematical models based on a deeper understanding of the principles involved. The inherent complexity of such formulations, as it may, at first sight seem to be their major disadvantage for practical applications, can be easily overcome with the use of modern digital computer techniques. The author's work follows the path of its predecessors in attempting to set up a realistic

and rational theory for the progression of BOD in streams.

The classical kinetic theory in this area stems primarily from the early works of Streeter and Phelps,[1] to whom the honour of its establishment is due. The first-order, monomolecular equation, they suggested for the graphic path of the carbanaceous BOD curve, stipulated that the rate of biochemical oxidation of the organic matter is proportional to the remaining concentration of the unoxidised substance. Their formulation was an enormous oversimplification of the reactions taking place in an environment characterised by very complex parameters - that were physical, chemical and biological in nature. Speculations were mainly raised against the extreme flexibility of the first-order equation which seemed to produce almost any conceivable type of curve with indefinite values of the constants. Small differences in the shape of the experimentally observed curve could produce great fluctuation in the computed values of the constants, so that little physical significance can be really attributed to them. The theoretical basis for the application of such an equation to biochemical oxidation was derived from the fact that many simple chemical diffusion and reaction phenomena follow the monomolecular pattern. However, no due recognition was paid to the fact that the biological oxidation of sewage and other pollutional organic matter involves a complex microbial flora which accomplish the oxidation by thousands of interrelated enzyme reactions occuring simultaneously during cell metabolism and multiplication. Thus the overall kinetics of

bacterial respiration are indeed more complex than is often realised. It is evident that the important facets of the BOD-kinetics are inexplicable by the first-order equation. In short, Streeter-Phelps formulation will only be a crude simplification for the case of streams receiving wastes from communities and industries.

Since the classical work of Streeter and Phelps, several modifications were suggested by investigators like Thomas[3], Fair[4], Oxford and Ingram[2] etc. Evidence has accumulated in recent years on the discordance between the experimentally observed curves and the theoretically established ones by the above-mentioned research workers. The most notable reason for this lack of correspondence can be easily traceable to the absence of a term in these equations representing the bacterial concentration. After all, it is the faculty of bacteria that is responsible for the removal of BOD and it seems unjustifiable to have denied its due importance in the BOD-equations. Actually, this has been acknowledged by Phelps himself.

Recent research works pursued by Busch[6] and Gaudy et al[7] have demonstrated the presence of more than one phase in the carbonaceous BOD curve. The diphasic curves they observed for different substrates under various seed conditions exhibited a "plateau" that separated the rapid phase of oxygen uptake from the slower phase. On the basis of their experiments, a theory was propounded which ascribed the progressive variation in BOD values to the effect of varying ratios of bacteria to higher

organisms in the seed population. Having established that the progression of BOD in soluble substrates was a two-stage and not a first-order reaction, they concentrated their efforts on the reproducability of the plateau and its subsequent reliability to suggest a short-term BOD test. However, some uncertainity still prevails regarding the existence of such a plateau since Butter field's observations[9] have expunged such an existence. Other important developments include the mathematical two-phase formulations of Garret and Sawyer[13] for bacterial growth.

The exact interpretation of the BOD kinetics confronts the engineers with many problems that have blanketed this area to a great extent in the past. (The problem is as analogous to that confronting mice who, in Aesop's fable, wondered how to hang a bell around the cat's neck to warn them of its presence). It is extremely a complicated task to propose any mathematical model, based on the kinetics of individual biochemical reactions involved in the exertion of BOD In the absence of a refined analysis, it becomes necessary to view the bacterial kinetics with a 'macroapproach' to gain atleast some insight.

The proposed model for BOD progression by the author, invokes the universally established Michaelis-Menten hypothesis for the rational justification of its formulation. It is well founded on the works of Monod[5] on bacterial growths in chemostats. The revelation of his works focussed considerable

attention of the research workers towards the deeper understanding of the principles involved in the progression of BOD. Recent experiments on the study of concentration effects in the biological oxidation of trade wastes by Wilson[15] have corroborated the conclusions derived by Monod. Wilson sought to extend the knowledge of basic principles towards the achievement of low-cost treatment systems operating at optimum biochemical efficiency.

Parallel to the Michaelis-Menten type of enzyme-catalysed reactions, the BOD equation can be written as,

$$\text{Bacteria} + \underset{\text{(Substrate)}}{\text{BOD}} \xrightarrow{\text{oxygen}} \begin{array}{l}\text{Increased number of bacteria} \\ + \\ \text{Oxidised products } (CO_2,\ H_2O,\ NH_3 \text{ etc.})\end{array}$$

Applying the laws of mass-action to the above equation, it is evident that the rate of BOD exertion will be proportional to the concentration of two substances viz., the enzyme (that is contained in the bacterial vessels) and the substrate. Even though a closer investigation of the metabolic activities of bacteria may reveal the involvement of a large array of enzymes, as a simplification, the concentration of bacterial-cell-mass can generally be considered to represent the total catalytic activity in the system.

The mathematical model presented by the author for the progression of BOD in streams emphasises the presence of a term in the BOD-equation to take care of the bacterial population. It incorporates as many constants as there are variables and

it is suggested that a compilation of BOD-curves for different conditions be prepared after solution of the proposed equations by digital-computer-techniques. Once this is done, the application of the derived formulations becomes facilitative for routine works. It only remains to aim for the proper match of the experimentally observed BOD-curves with the theoretical graphs.

To sum up. The model is rational because it recognizes the due significance of the role played by bacteria in the biological purification of wastes, dumped to the streams; It has got sound bearing in the annals of enzyme - chemistry of catalysed reactions; It describes adequately the full gamut of BOD-kinetics; Its application is more direct and facilitative than that of a first order equation. The author has undertaken experiments to validate the theory proposed. It is sincerely hoped that due consideration will be given towards the application of such realistic and rational formulations by those who want to lean on more scientific approaches than on the existing crude formulations while pursuing river-pollution-abatement programmes.

b. Scope and Object of Study:

As already indicated in the previous section, presentation of such a model as to adequately describe the BOD progression in streams based on a deeper understanding of the bacterial kinetics involved, has been the main aim of the author. Efforts have also been taken for a comparison of the rational model with the Streeter-Phelps-equation, primarily to bring out the inadequacy of the later formulation in dealing with the practical situations.

The study, in short, consisted of the following two parts:

1. To formulate a rational model for the BOD progression in streams and demonstrate its use in the practical problems of river-pollution-evaluation.
2. To undertake experimental studies to look for agreement with the proposed theory.

The numerical solutions of the mathematical formulations are to be worked out with the help of digital computer and their graphic paths, traced. It is easy to compile the different sets of curves for different values of the constants, under various initial conditions. The author, however, refrains from doing so as the work will otherwise become quite voluminous. He prefers to demonstrate the use of some 'model' graphs and he would like to pinpoint the necessity of preparing the various sets of BOD-graphs by the concerned agencies in the area of river-water-pollution-control.

CHAPTER II

REVIEW OF LITERATURE

The major findings of the early works on BOD kinetics, can be, for better understanding, categorised in the following main three groups:

1. Those, which were reported as modifications to the Streeter-Phelps - first order equation that suffered very much from its extreme flexibility in the determination of the constants. The investigators of this group attempted to strike at a close correspondence of the experimental data with the theoretical ones. Of notable mention are the "lag formula" developed by Thomas[3], the "retardant" BOD equation formulated by Fair[4] and the "logarithmic BOD equation" suggested by Oxford and Ingram.[2] Their works revealed only one phase in the carbanaceous BOD curve.

2. Those of very recent times, that describe a new type of kinetics for the course of carbanaceous BOD comprising more than one phase. Research works of Busch[6] and Gaudy et. al[7] are of notable mention in this connection. They investigated the influence of the higher form of microorganisms on BOD progression of soluble substrates and reported the presence of a "plateau" or discernible pause separating the earlier rapid phase of oxygen uptake from the second stage. Based on the diphasic formulation, Busch suggested a short-term BOD test (T_b oD) that involved tracing the BOD curve to the plateau and determining the cell oxygen equivalent at that point. It should also be noted however that not

the geometry of BOD curves has not yet been defined with enough precision to warrant the use of plateau for predicting the course of BOD removal. Also, Butterfield's experiments (and incidentally the experiments of the author too) have showed no evidence of the occurrence of such plateau in the carbanaceous BOD curve. Thus there lies still a great degree of discrepreaparing or atleast inconsistency regarding the occurrence and implicatbons of the plateau.

3. Those, which interpret the kinetics in a mathematical fashion and are of particular significance for practical considerations. They are well founded on the theories already established (or) atleast on the observations, frequently noticed, of the two main methods of representing the kinetics of the biochemical reactions involved in the BOD progression, one was due to Monod, which was subsequently used by Hinshel wood [10] Herbert[11], Downing[12] and others. It relates the growth rate of the active organisms(which is a measure of rate of purification) by the well known Michaelis-Menten relationship that will be given later. The second proposed by Garret and Sawyer[13] and adopted by Eckenfelder and McCabe[14] postulates two growth phases - one in which the substrate concentration is always greater than a threshold value required for the complete saturation of the active enzyme centers of the organisms, the other in which the degree of unsaturation increases as the substrate is progressively utilised as food. These two postulates still require intensive data collection for their unwarranted recognition in their adoptability for the highly engineered works in

the field of biological systems. Driven by lack of confidence in the existing kinetic concepts, I.S. Wilson[15] undertook special experimental studies the result of which he interpreted in the light of both the Monod and the two-phase theories. His published data fitted well the Monod's theory. Clarks et al[16] and Charles S. Revelle[17] were fasciated towards second order BOD equations. Very recently, a stochastic model for BOD and DO in streams has been presented by Richard P. Thayer et al.[18]

Since the author's work comes under group - 3, mention should be made in brief the methods of approach of the various investigators of that group in particular, towards the v rational considerations of the kinetics involved.

In 1942, Monod[5] conducted a quite complex study of the growth of pure cultures of E. Coli and B. Subtilis on an assortment of single carbohydrate substrates. He was able to fit his data very closely to the rate concentration curve of the same form as the Michaelis-Menten equation for the rate of enzymatic reactions, viz.,

$$\mu = \frac{K_{max}\ S}{(K_S+S)} \qquad \text{where,}$$

μ = growth rate of cells

K_{max} = maximum growth rate when the substrate is unlimited.

K_S = substrate concentration at which the growth rate observed is one half of the maximum value; saturation constant.

S = Concentration of the substrate

Verifications of Monod's conclusions were done by Mr. Andrew L. Gram[19] in 1956. After conducting extensive experiments, Gram found that the rate of removal of substrate was closely described by Monod equation. He, as well as Stewart and Ludwig[20] noted that the removal rate per unit weight of organisms was a function of substrate concentration only if a constant conversion of substrate to organisms was assumed.

Earlier, in 1952, Garret and Sawyer[13] postulated that there were only two phases- a log phase and a transition to the stationary growth phase - that were of "practical" importance in defining the reaction kinetics of aerobic biological processes. Even though they agreed that Monod's equation was well founded theoretically, they declared that the observed data were in better agreement with the two-phase formulation than with Monod's equation. Their main objection to Monod's relationship was that "the equation denies the existence of a constant rate of growth above critical concentrations of food, although this is the most frequently observed phenomenon related to the growth of bacteria." The two phase formulation for bacterial growth was comprised of the following two equations:

In Phase I,

$$\frac{dX}{dt} = K_1 X$$

In Phase II,

$$\frac{dX}{dt} = a K_2 X S$$

where, X = Bacterial concentration.

S = Substrate concentration

a = Increase in organism concentration produced by unit decrease in substrate concentration.

K_1 and K_2 = the rate constants.

The results of Garret and Sawyer also showed that the kinetics of oxygen utilisation by mixed organisms were similar to those for pure cultures, although the rates were lower.

Sometime in 1962, I.S. Wilson[15] undertook extensive lab studies to seek refinement of the theories of Monod and Garret and Sawyer. He tried to evaluate the constants in their equations with notable success. His main concern was in the practical utility of these equations towards the development of high-efficiency, low-cost biological-waste-treatment-systems. He emphasised the need for rigorous control of experimental conditions, for even slight errors in fixing one or two points in the rate curve would cause large error in the interpretation of the results. His published data fitted better the following Monod-Type-equation than the two phase formulation.

$$\frac{dS}{dt} = \frac{K_X K_{max} X S}{(K_S+S)}$$ where,

K_X = the reciprocal of "yield coefficient" which is the increase in the cell concentration per unit substrate-depletion.

(K_X, K_{max}, X and S denote the same quantities as defined earlier.)

The major limitation of his paper, as pointed out by Mr. P.H. McGauhey[21], was that he did not make any conclusions particularly evident.

In 1964, Keshavan et al[22] conducted studies on the kinetics of removal of organic wastes, in connection with their proposed rational formulations. The correlation between the experimental data and the theory was very good. They invoked the well established Michaelis-Menten hypothesis for the justification for their rational formulations.

The second-order BOD equation suggested by Clark et al[16] in April 1965, was of the following form:

$$-\frac{dS}{dt} = K S^2$$ where,

K is the rate constant

This was based on the following reaction,

$$2\,S \longrightarrow \text{Products.}$$

i.e. $$2\,(BOD) \rightarrow \text{Products (like } CO_2,\ H_2O,\ NH_3 \text{ etc.)}$$

With their experimental evidence they concluded that " a second-order BOD equation is as good a fit as the first-order equation for the observed data" and that "a second-order equation can be solved with more facility than can an equation based on a first-order reaction." Challenging the rationality of their model, Keshavan et al[23] severely objected that the model could not be

justified even if the Michaelis - Menten hypothesis was invoked by the authors. Their main argument was that the rate of reaction should be proportional to the concentration of two substances, namely the substrate and the enzyme. Clark et. al, they concluded, were not at all justified to have proposed the above equation.

The works of Keshavan et al mentioned earlier, contributed significantly towards the development of second order Bio-oxidation kinetics by Charles S. Revelle et al[17] in December 1965. The latter suggested that the rate of BOD removal was proportional not only to the concentration of BOD remaining, but also to the concentration of bacteria, each raised to some power, as follows:

$$\text{Rate of BOD removal} = \left[\text{Concentration of BOD remaining}\right]^{n} \left[\text{Concentration of Bacteria}\right]^{m}$$

Since the exact determination of the values of n and m in the above equation remains still a puzzle with the present knowledge of enzyme kinetics concerning BOD reactions, the value of n and m was taken as 1 in order that the equation may be amenable for further mathematical treatment. In the period before the onset of endogenous respiration, their second order equation provided a realistic and rational approach to the kinetics of biological oxidation.

The stochastic model for BOD in steams presented recently by Richard P. Thayer et al[18], assumes, however, a first-order reaction-rate for the organic-pollution-removal.

The literature study brings out clearly the necessity of isolation of an area where added research is highly needed before certain current postulates can be used with confidence. The area

not just the formulations, but their widely recognised acceptance by many for their useful applications. This calls for a deeper understanding of the basic principles involved. The difficulties that surmount the accurate prediction of kinetics in this area need not be cataloged here, as they are the ones, very often encountered in association with any biochemical reaction involving thousands of enzymatic systems at intra-cellular levels. Thus the inherent complexity that has blanketed the area is to a great extent unavoidable unless there is a major break through by investigators who will propose methods for the exact determination of the order and molecularity of the complicated, deeply involved biochemical reactions. However it is also possible to gain sufficient insight, in the light of the existing knowledge, towards the formulation of rational expressions well-founded on basic theories and adequately supported by practical observations.

CHAPTER III

THEORETICAL CONSIDERATIONS

a. Basis of BOD Determinations:

The ultimate foundations underlying the biochemical oxygen demand of waste materials are the enzyme catalysed processes involved in the growth and multiplication of organisms acting on these materials. Oxidation, as it applies to biological and chemical processes, may be described as the loss of electrons from a substrate. The primary biological function of oxygen in aerobic systems is that of a terminal acceptor for electrons liberated by oxidation reactions carried out during cell metabolism. The electrons are sequentially transfered along a respiratory or 'electron transport' chain through the coupled cyclic action of several carrier systems to oxygen, the terminal acceptor, yielding water as an end product. The 'cytochrome chain' includes the substrate, NAD^{+}, flavoproteins, several cytochromes and the terminal acceptor, oxygen. Hence it is reasonable to expect that the uptake of oxygen during cell growth should be in direct proportion to the sum of all the oxidative processes occuring within the cell. As it is known that the extent of cell growth is directly proportional to the quantity of biologically oxidisable substrate present, it is possible to indirectly assay the 'strength' of the waste materials by measuring the quantity of oxygen uptake by the system. This is the basis of BOD determina-

tions. Further it is well understood that the rate of oxygen uptake is a function of the rate of substrate oxidation by the cell, and hence, ultimately traceable to the rate of enzyme reaction.

b. Order and Molecularity of Reactions:

Before considering the kinetics of BOD reactions, the salient features of ordinary or 'uncatalysed' reactions shall be reviewed with specific reference to their order and molecularity.

In accordance to the law of Mass Action, 'the rate of reaction at each instant is proportional to the concentration of reactions; each concentration being raised to a power equal to the number of molecules of that reactant participating in the process'. 'Kinetic order' refers to the sum of the exponents of the concentrations which determine the reaction rate. 'Reaction Velocity' refers to the time rate of change concentration of the compound considered. This denotes generally the rate of reactant disappearance, whilst, in enzyme reactions of biochemistry, it applies to the rate of product formation. It is to be pointed out that for reactions with no intermediate products, the rate of reactant disappearance is the same as the rate of product formation, where the stoichiometric ratio of reactant to product is unity.

In general for a reaction involving q, r, ... molecules of compounds A, B, . . . , the rate equation is

given as follows:

$$qA + rB + \ldots \xrightarrow{K} \text{products}$$

$$v = -da/dt = K\, a^q\, b^r \ldots\ldots$$

where a & b . . . are the respective concentration of A, B, . . .

The reaction order, $n = q + r + \ldots$.
Here the n - molecules must interact with each other in the rate-limiting step of a reaction, and the rate equation for this n-th order reaction contains n - concentration terms, and the rate constant has the dimensions of concentration$^{(1-n)}$ time^{-1}. It should be however borne in mind that, it is statistically highly improbable that all the n - molecules participate simultaneously in a rate-limiting step. They may proceed, by a series of any reaction order. For example, a termolecular reaction may proceed by a series of bimolecular, and/or monomolecular steps. Reactions of this type are kinetically of first, second, third or mixed over-all - depending on which steps are rate-limiting, and even fractional orders are not uncommon.

For example, the reaction $3\ KClO_2 \longrightarrow KClO_3 + 2\ KCl$ will be of 3rd order, if molecularity were to equal order. But, actually this is a second order reaction in KClO.

From the foregoing, it is evident that the determination of the order of a reaction is the most important part in describing its kinetics and that order of a reaction need not equal molecularity. In the following discussions, considerations will be given

to the theoretical order and molecularity of BOD reaction.

c. Kinetics of BOD Reactions:

1. General Observation:

The BOD reaction can be described as,

$$\text{Organic Matter (or BOD)} \xrightarrow[+\ O_2]{\text{bacteria}} \text{Oxidised products } (H_2O,\ CO_2,\ NH_3) \text{ etc.}$$

For illustrative purposes, let an organic compound, glucose be considered. Oxidation of glucose yields CO_2 and water. This can be represented as:

$$C_6H_{12}O_6 \xrightarrow[+\ O_2]{\text{bacteria}} 6\ CO_2 + 6\ H_2O \qquad (1)$$

But the actual sequence leading to the formation of products is far from simple. Phosphoenol pyruvate which is formed via the glucolysis path way, enters the Krebs cycle in the form of Acetyl CoA. The Krebs cycle is as complex as the preceeding steps. Thus the equation 1 is just a summation of the thousands of inter-related enzyme reactions occuring simultaneously during cell metabolism and multiplication. It becomes clear that the over-all kinetics of bacterial respiration are more complex than is often realised. A more extensive discussion of enzyme reaction mechanisms may be found in the work of Reiner[24].

From the previous discussions, it is quite evident that the oxidation of glucose by the bacterial cells can not be a first order reaction, because of the number of intermediates

between glucose and the final step. Each intermediate step has its own orderef of reaction and involve different enzymes. Further the total enzyme concentrations of the system increase with the replication of the bacterial cells. Hence one must admit the lack of knowledge of molecularity, and inability to test the order of BOD reactions, before proposing any rational model. When such is the case for only one substrate like glucose, it is not difficult to realise the degree of enormous complexity involved in predicting the exact kinetics of the biochemical oxidation of other heterogeneous compounds.

2. Michaelis - Menten Hypothesis Applied to BOD Reactions:

With the dearth of knowledge already admitted, regarding the order and molecularity of the BOD reactions, one has to take a 'macroscopic' approach to describe the BOD kinetics. Since the BOD - reaction is entirely biochemical, the Michaelis-Menten hypothesis can be invoked for a formulation of a similar expression. An enzyme - catalysed reaction of Michaelis-Menten type can be represented as follows:

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} E\,S \xrightarrow{K_3} P + E \qquad (2)$$

Where E is the enzyme, S represents the substrate, E S denotes the enzyme - substrate complex and P stands for the product. K_1, K_2, and K_3 are the rate constants in this reaction.

If the value of K_3 is far less than that of K_1, i.e. if the rate of product formation is controlled by the specific

rate K_3 in the sequence of reactions expressed by equation (2), the following can be written.

$$v = \frac{K_3 \; E \; S}{K_M + S} \qquad (3)$$

where v is the rate of reaction.

K_M - the Michaelis - Menten Constant.

E - the enzyme concentration

From (3) it is clear that the rate is reaction is thus dependent on the concentration of two substances, namely ~~vis~~ the enzyme and the substrate.

In the same fashion, considering the concentration of the bacterial to represent the enzyme concentration (at least in a 'macroscopic' way), the macro-reaction for BOD would be written as:

$$\text{Organic matter (Substrate)} + \text{Bacteria} \xrightarrow{\text{Oxygen}} \text{More Bacteria} + \text{Oxidised products.}$$

In light of the 'previous discussions, the following second-order bimolecular reaction would be written for BOD exertion.

$$-\frac{d}{dt}(\text{BOD}) = K \; (\text{BOD}) \; (\text{Bacteria}),$$

each quantity within the brackets representing respective concentration.

It was this form of BOD expression, which Charles S. Revelle et. al proposed and their experimental results revealed a good striking coincidence with the stipulated model.

d. The Rational Model:

1. Mathematical Formulations:

The rational model has been based mainly on Monod's results (on bacterial growth in a chemostat), that behaved in a manner typified by Michaelis-Menten equation. The expression he suggested for the bacterial growth was of the following form:

$$\mu = \frac{K_{max}\ S}{K_S + S} \qquad (4)$$

Where μ - growth rate of alls $\frac{d}{dt}(\ln x)$
(i.e. $\mu = \frac{1}{x}\ \frac{dx}{dt}$)

K_S - substrate concentration at which the growth rate observed is one half the max value; saturation constant.

X - Bacterial Concentration at time t

S - Substrate concentration at time t.

Equation (4) can be written as:

$$\frac{dx}{dt}\ \frac{1}{x} = \frac{K_{max}\ S}{(K_S + S)}$$

or,

$$\frac{dx}{dt} = \frac{K_{max} \; X \; S}{(K_S + S)} \qquad (5)$$

Assuming that an increase in the bacterial mass is taken as directly propertional to the amount of BOD removed, it can be written as,

$$(X - X_o) = (S_o - S) \frac{1}{K_X} \qquad (6)$$

Where, K_X is the reciprocal of the well known 'yield coefficient'

X_o = Initial bacterial concentration.

S_o = Initial substrate concentration.

Differentiation of (6) with respect to the third variable, time, denoted by 't', leads to,

$$\frac{dx}{dt} = - \frac{ds}{dt} \quad \frac{1}{K_X} \qquad (7)$$

and substitution for dx/dt from equation (5) in equation (7) results in the following equation, describing the kinetics of BOD progression.

$$- \frac{ds}{dt} = \frac{K_X \; K_{max} \; X \; S}{(K_S + S)} \qquad (8)$$

Equation (8) is also the same form of the equation adopted by Wilson.

Equation (8) is a second-order differential equation in S. It contains 3 variables, viz. S, X and t. Substitution for X from equation (6) in eqn. (8), permits integration of the later equation in only two variables S and t, as follows:

$$-\frac{dS}{dt} = \frac{K_X \quad K_{max}}{K_S + S} \left(X_o + \frac{1}{K_X} (S_o - S) \right)$$

i.e.

$$\frac{-\, dS\ (K_S + S)}{(X_o + \frac{1}{K_X} (S_o - S)} = K_X \quad K_{max} \quad dt$$

Integrating both sides, within appropriate limits,

$$\int_{S_o}^{S} \frac{-\, dS\ (K_S + S)}{(X_o + \frac{1}{K_X} (S_o - S))} = K_X \quad K_{max} \int_{o}^{t} dt \qquad (9)$$

Equation (9) takes the final form after *integration as follows:

$$S = S_o \left\{ 1 + (X_o/S_o) \quad K_X - (K_S/S_o)\ K_X \left[\frac{e^{K_X \quad K_{max} \quad t}}{\left\{ \frac{X_o + \frac{1}{K_X} (S_o - S)}{X_o} (S_o/S) \right\} \frac{K_S}{X_o + \frac{1}{K_X} S_o}} \right]^{\frac{1}{K_X}} \right\} \qquad (10)$$

* Integration is shown in the appendix.

Equation (10) is the proposed kinetic expression for the substrate depletion in streams. It will be recalled that the constants K_{max} and K_S go to describe adequately the rate behaviour of the BOD reactions with the initial conditions given by S_o and X_o.

Equation (5) describing the bacterial growth can be invoked again for further mathematical treatment. Substituting in this equation the value of S from equation (6), the following differential equation is obtained.

$$\frac{dX}{dt} = \frac{K_{max} \; X \; (S_o - K_X \; (X - X_o))}{(K_S + S_o - K_X \; (X - X_o))} \qquad (11)$$

Rearranging equation (11) and *integrating it between the limits (t 0, X X_o, t t, X X) the following equation showing the relationship of bacterial concentration with time.

$$X = X_o \left[\left\{ \frac{S_o + K_X \; (X_o - X)}{S_o} \right\} \frac{X_o}{S_o + K_X \; X_o} \cdot e^{K_{max} \; t} \right]^{\frac{S_o + K_X \; X_o}{S_o + K_X \; X_o + K_S}} \qquad (12)$$

Equations (10) and (12) which characterise the kinetic pattern of BOD progression and bacterial growth respectively, are amenable to solution by digital computer. Thus a compilation of different sets of graphs for various values of the constants under given initial conditions can be prepared. The experimentally observed graphs for BOD progression can be compared with the theoretical ones, and the prediction of river-pollution is made possible to a remarkably degree with the knowledge of the constants, K_S, K_{max} and K_X.

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* Integration is shown in the appendix.

2. Significance of the Constants:

Much of the practical significance of the proposed mathematical formulation is gained from the presence of the three constants viz., K_X (the reciprocal of "yield coefficient"), K_S (the saturation constant) and K_{max} (the max-growth rate constant). These three constants, along with the initial conditions X_o and S_o describe the organic-pollution - profiles in a stream in a more realistic manner than the constants of the first-order reaction do.

K_X is the reciprocal of "yield coefficient." Thus $1/K_X$ refers to the amount of bacterial-mass that has been assimilated in the depletion of unit amount of substrate. The assumption of the linear relationship between the bacterial mass and the amount of BOD-removed has been held valid by many research workers. [25, 26, 27, 28]. K_X as one will expect, remains constant for a given substrate and organism, and is dimensionless.

K_{max} and K_S are the two constants, standing for the kinetic properties of bacterial reactions. K_{max} is the maximum value of growth rate at infinite substrate concentration (i.e. $S \rightarrow \infty$) and thus has the dimensions of inverse time. It has thus a well defined meaning both operationally and physically.

The saturation constant, K_S, can be stated mathematically as follows:

$$K_S = \left[(S)\right]_{\mu} = \frac{K_{max}}{2}$$

i.e. it equals the concentration of the substrate, S at which the observed growth rate is half the maximum growth rate. Both the constants K_X and K_{max} vary with the organism and substrate but do not seem to be dependent on temperature to a signfficant degree at maximum growth rates.[5]

3. Assumptions in the Model:

In view of the fact that the difficulties towards the development of a comprehensive kinetic theory for bacterial oxidation can not be easily surmounted with the existing knowledge in that area, the proposed model by the author leans heavily on the following assumptions that are compulsory.

A principal assumption is that the total catalytic activity of the system is represented by the bacterial concentration. When a bacterial cell multiplies, all of its constituents are assumed to be identically reproduced and hence the enzymic concentrations increase in direct proportion to the increase in bacterial mass.

The kinetics of mixed culture is considered as though the mixed culture were pure. This assumption has been already validated by Garret and Sawyer.[13]

Additionally, an increase in the bacterial mass is taken as directly proportional to the amount of BOD removed. This has been verified earlier by many research workers.

Finally, oxygen concentration is not a limiting factor, i.e. there is enough supply of oxygen. But for this assumption, the mathematical treatment of the model would have become much involved.

It was also assumed that the variables like temperature, pH etc. are maintained at an optimum level in the environment and that the supporting medium contains all the essential growth factors and no inhibiting substances.

CHAPTER IV

EXPERIMENTAL METHODS

a. Experimental Techniques for BOD Determination:

The experimental data for BOD were obtained with the Warburg Techniques rather than with the standard dilution techniques. A number of factors were responsible for this choice. The standard dilution test requires a large number of BOD bottles because of short time intervals (4 hours), whereas only one manometer and flask will be needed for each dilution of the seed in the Warburg method. Also the BOD curve can be followed in the early critical stages very easily and accurately by the manometric techniques. The shaking of the reaction flasks was kept minimum, so that this will , to some degree, correspond to the normal stirring in streams.

b. Measurement of Bacterial Growth:

For measuring the bacterial growth, optical density which measures in turn the bacterial density was made use of in the experiments. Bacterial density is defined as the dry weight of bacteria per unit volume of the solution. It is more closely related to the quantity of the bacterial protoplasm and hence to the enzyme activity. Optical densities for this study were measured on B & L Spectronic - 20 at an optimum wave length of 590 m u. The calibration curve for bacterial concentration is shown in Appendix A. Increasing concentration of the bacterial

cells for calibration purposes were obtained by centrifuging the domestic seed at 2×10^6g.

c. Substrates Used in the Experiments:

The substrates that were employed in the experiments were glucose, peptone and domestic sewage. These three are different kinds of wastes - glucose, a carbohydrate-waste, peptone, a proteiniceous - waste and domestic sewage, an extremely complex waste. The concentrations of the different substrates in the experiments are shown in Table I.

d. Seeding Conditions:

Domestic seed was employed in the experiment in the concentration of 2.5 mg/lit., 5 mg/lit., and 10.0 mg/lit. Inoculation of the domestic seed in the reaction-flasks ensured a hetrogeneous microbial population to act on the different substracts. The ratios of seed to substrate (R) to study the effect of bacterial concentration on BOD exertion were 1/8, 1/4 and 1/2. The seed was stored in the frigidaire through the entire period of experimentation (3 months about).

e. Sets of Experiments:

The experiments were conducted in Warburg-flasks of 125 ml capacity. The various sets of experiments are summarised in Table I.

TABLE I

SETS OF EXPERIMENTS

Substrate	Initial substrate concentration (mg/l)	Seed concentration employed (mg/l)	Ratio of initial concentration of Seed to initial Substrate Concentration R	Number of sets
Glucose	400	2.5	1/160	3
		5.0	1/80	
		10.0	1/40	
	240	2.5	1/96	
		5.0	1/48	
		10.0	1/24	
	120	2.5	1/48	
		5.0	1/24	
		10.0	1/12	
Peptone	400	2.5	1/160	3
		5.0	1/80	
		10.0	1/40	
	240	2.5	1/96	
		5.0	1/48	
		10.0	1/24	
	120	2.5	1/48	
		5.0	1/24	
		10.0	1/12	
Domestic Sewage	182 (as BOD)	2.5	1/72	1
		5.0	1/36	
		10.0	1/18	
				7

Note

(1) Dilution water used in the experiments to make up the total volume (of substrate, seed) to 50 ml was prepared according to Standard Methods.[28]

(2) Temperature of Thermostatic bath = $25^{\circ} \pm 1^{\circ}$ C.

CHAPTER V

EXPERIMENTAL RESULTS

The data obtained from the experimental studies on the kinetics of BOD exertion and bacterial growth for different substrates (Glucose, Peptone and Domestic Sewage) are presented in Appendix - B. The corresponding graphical plots are exhibited in figures 1 to 7.

The terms 'viable cell mass' and 'bacterial concentration' have been synonymously used by the author in all his works.

The symbol 'R' marked on the graphs is the ratio of the initial concentration of the seed to the initial substrate concentration and hence is a dimensionless number. This is a very significant parameter syggested by the author for river pollution studies and finds its frequent mention accordingly in the following chapters.

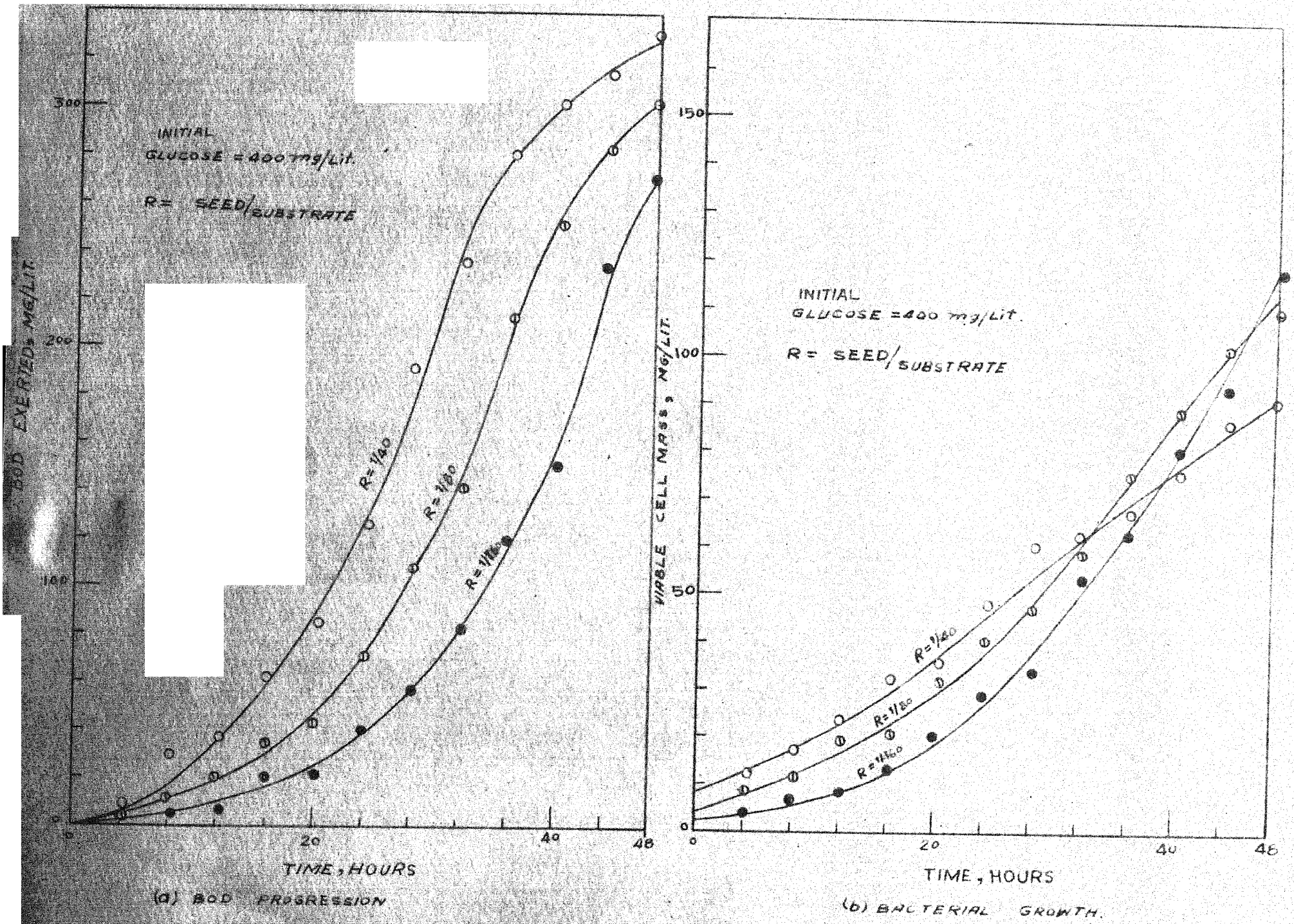


FIG 1 - OBSERVATIONS FOR SET (1)

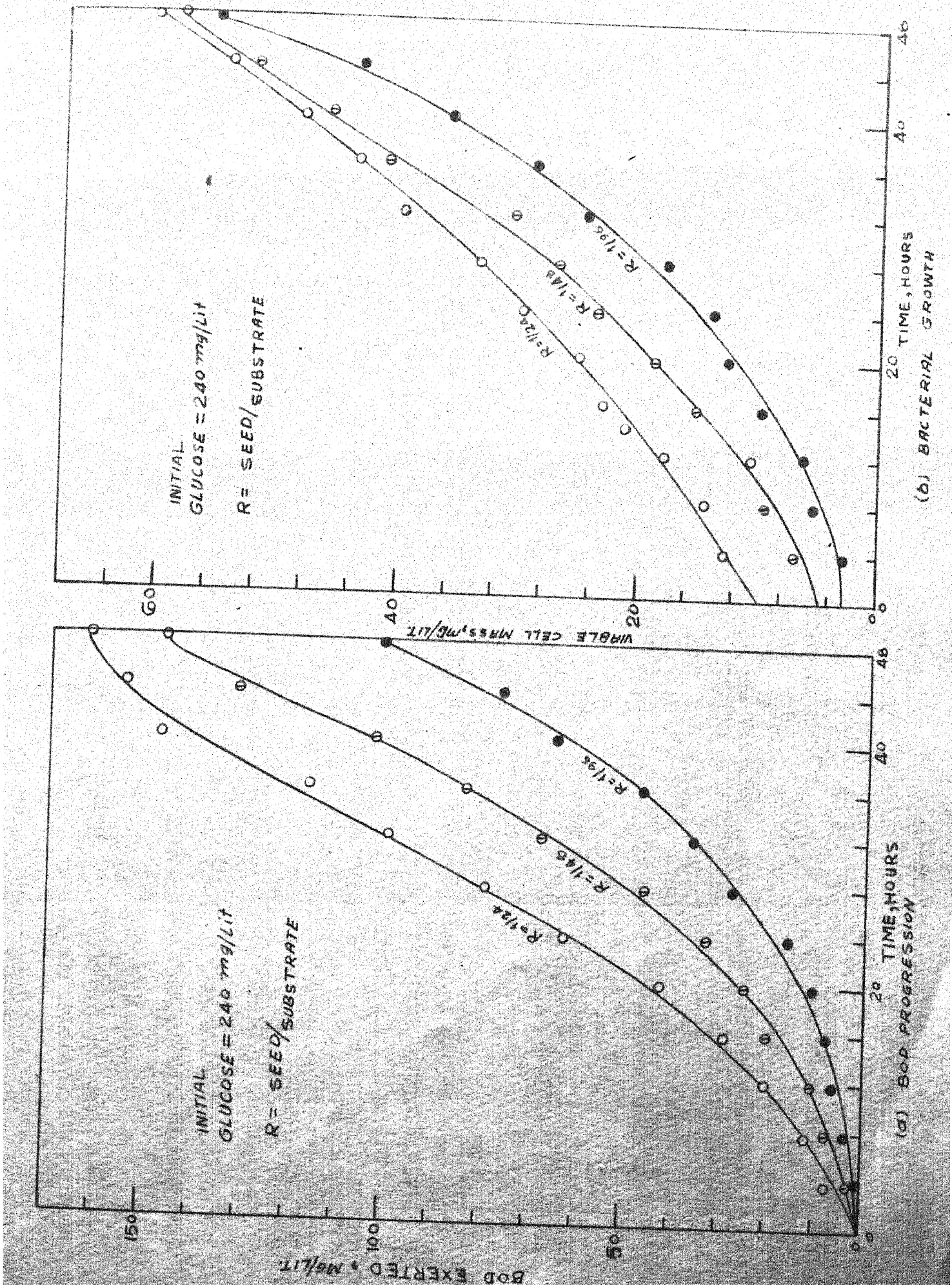


FIG.2:- OBSERVATIONS FOR SET (2)

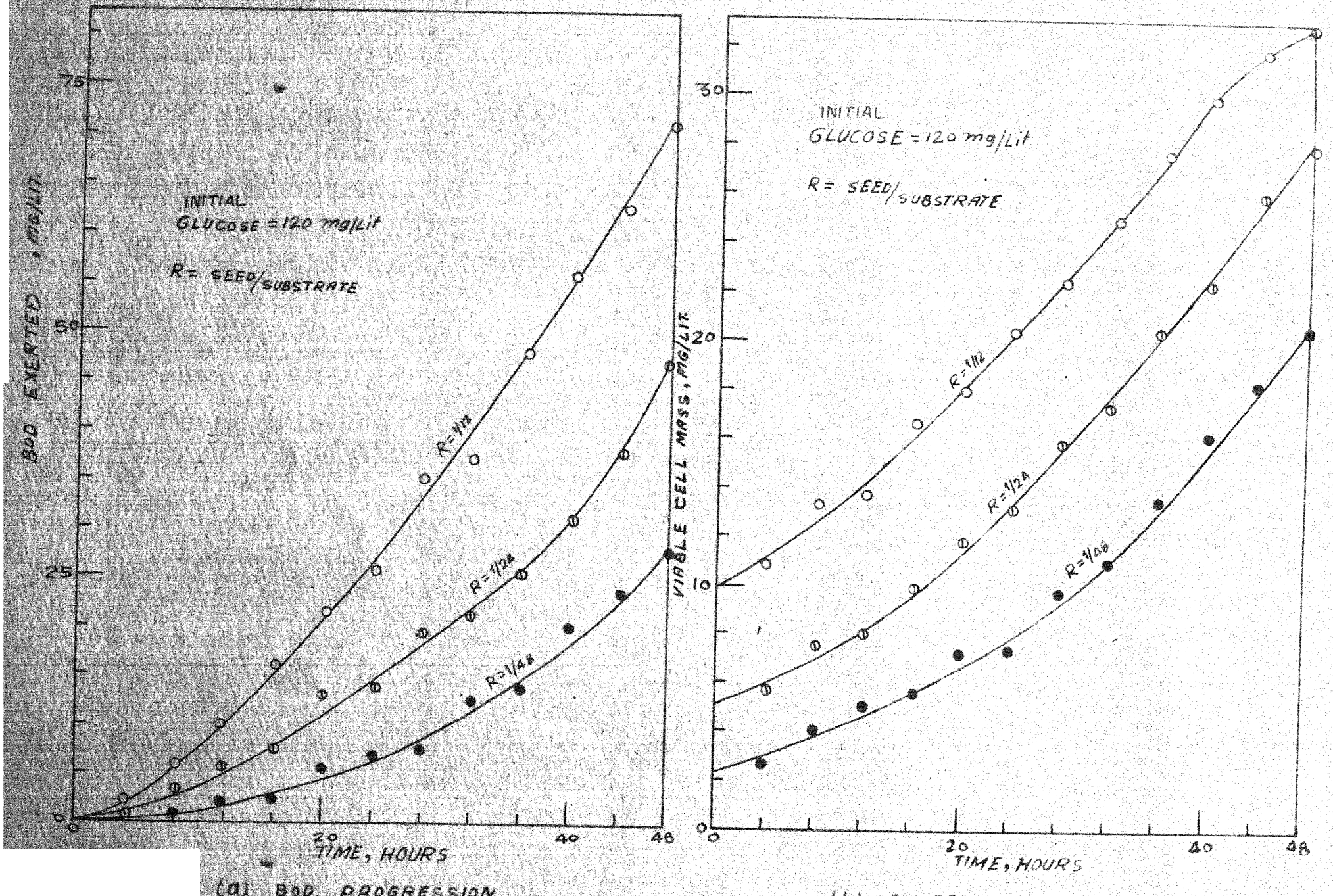


FIG. 3:- OBSERVATIONS FOR SET (3)

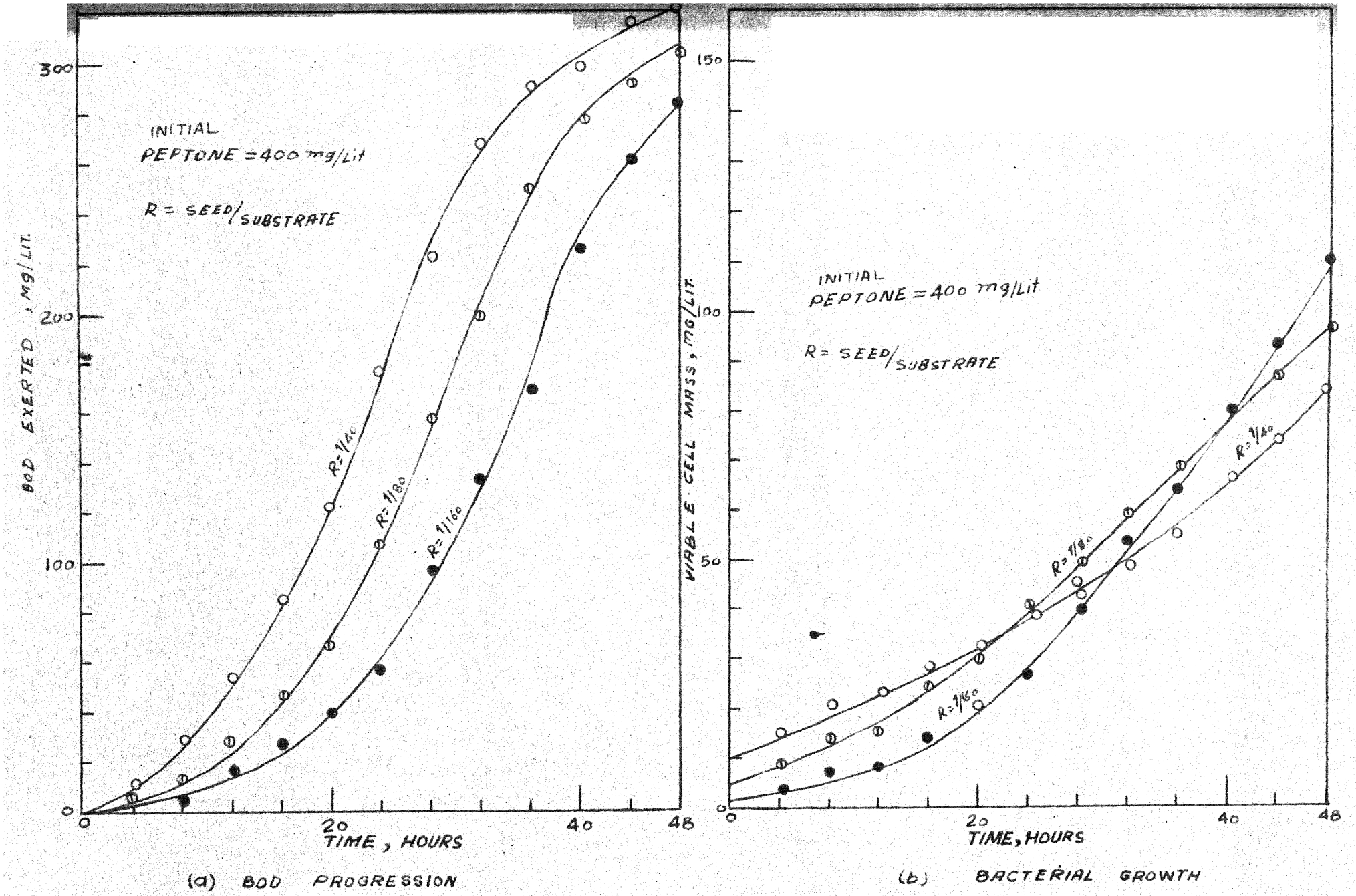


FIG.4:- OBSERVATIONS FOR SET (4)

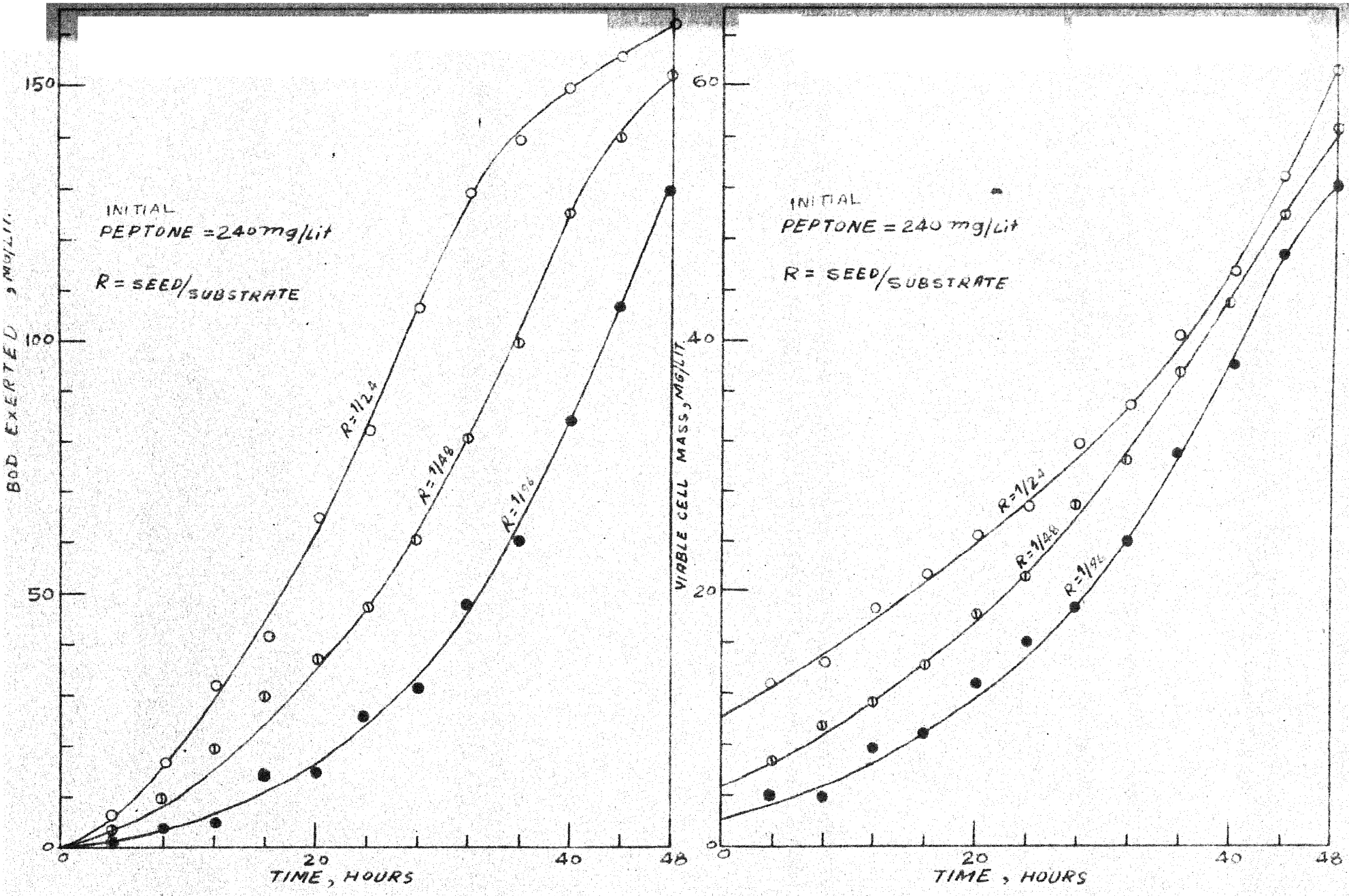


FIG. 5:- OBSERVATIONS FOR SET (5)

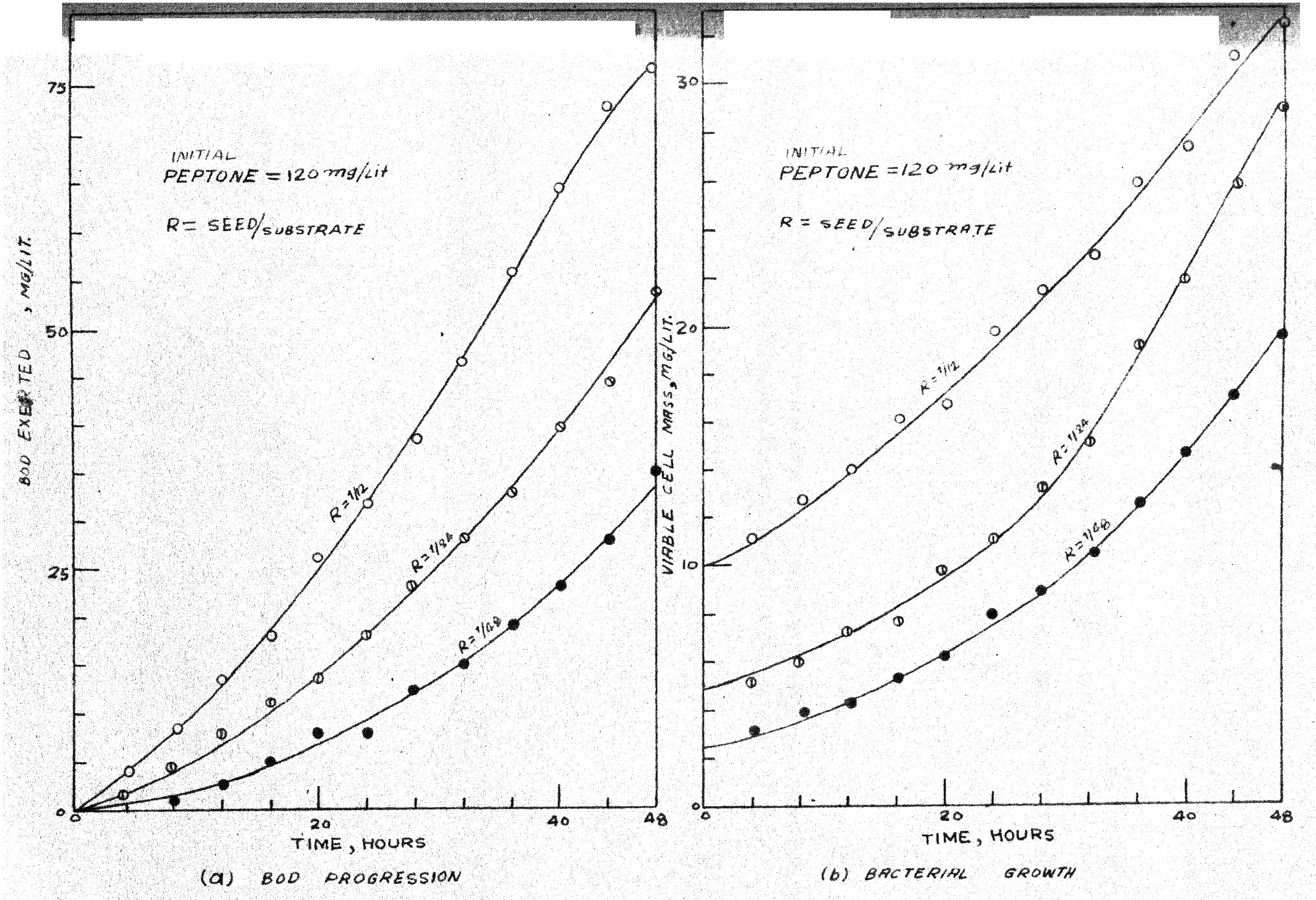
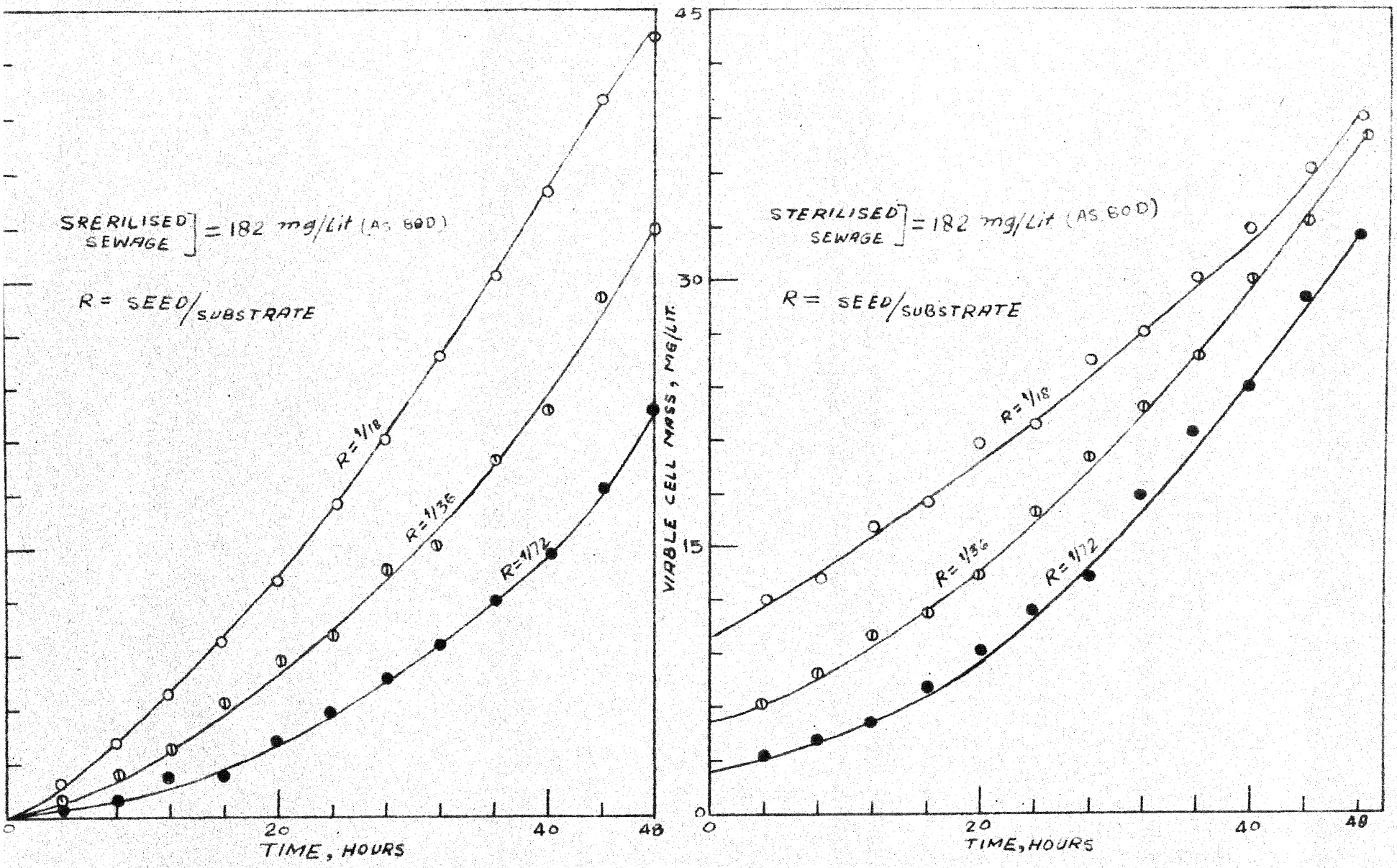


FIG.6:- OBSERVATIONS FOR , SET (6)



(a) BOD PROGRESSION

(b) BACTERIAL GROWTH

FIG.7 :- OBSERVATIONS FOR SET (7)

CHAPTER - VI

DISCUSSION AND ANALYSIS OF RESULTS

a. Theoretical Curves for BOD Progression:

The mathematical model was programmed for computer operation and the numerical solutions were worked out for arbitrarily chosen values of the constants (K_S, K_{max}, K_X) under assumed initial conditions (X_o, S_o). These values are plotted in Figures 8 to 12 which demonstrate clearly the kinetic course of the substrate depletion according to the proposed theory. Casual observation of these graphs will atonce reveal their significant departure from the path of the Streeter-Phelps formulations. The effect of variation in the values of the constants K_S, K_X and K_{max} on the geometry of the curves can be easily studied from graphs 8, 9 and 10 respectively. It is interesting to note that this effect is more pronounced for changes in the values of kinetic constants K_S and K_{max} than for the constant K_X. Even though K_X changes from 1.5 to 2.5, (i.e. yield coefficient changing from 0.66 to 0.40), there is no much noticeable effect on the rate of utilisation of substrate by the microorganisms. As expected from the analysis of the equation of the model, the decreasing values of K_S result in a faster substrate-consumption and the effects are just the opposite for similar changes in K_X and K_{max}. Figures 11 and 12 present the graphic evidence for the effect of variation in different initial conditions (X_o and S_o) on substrate depletion .

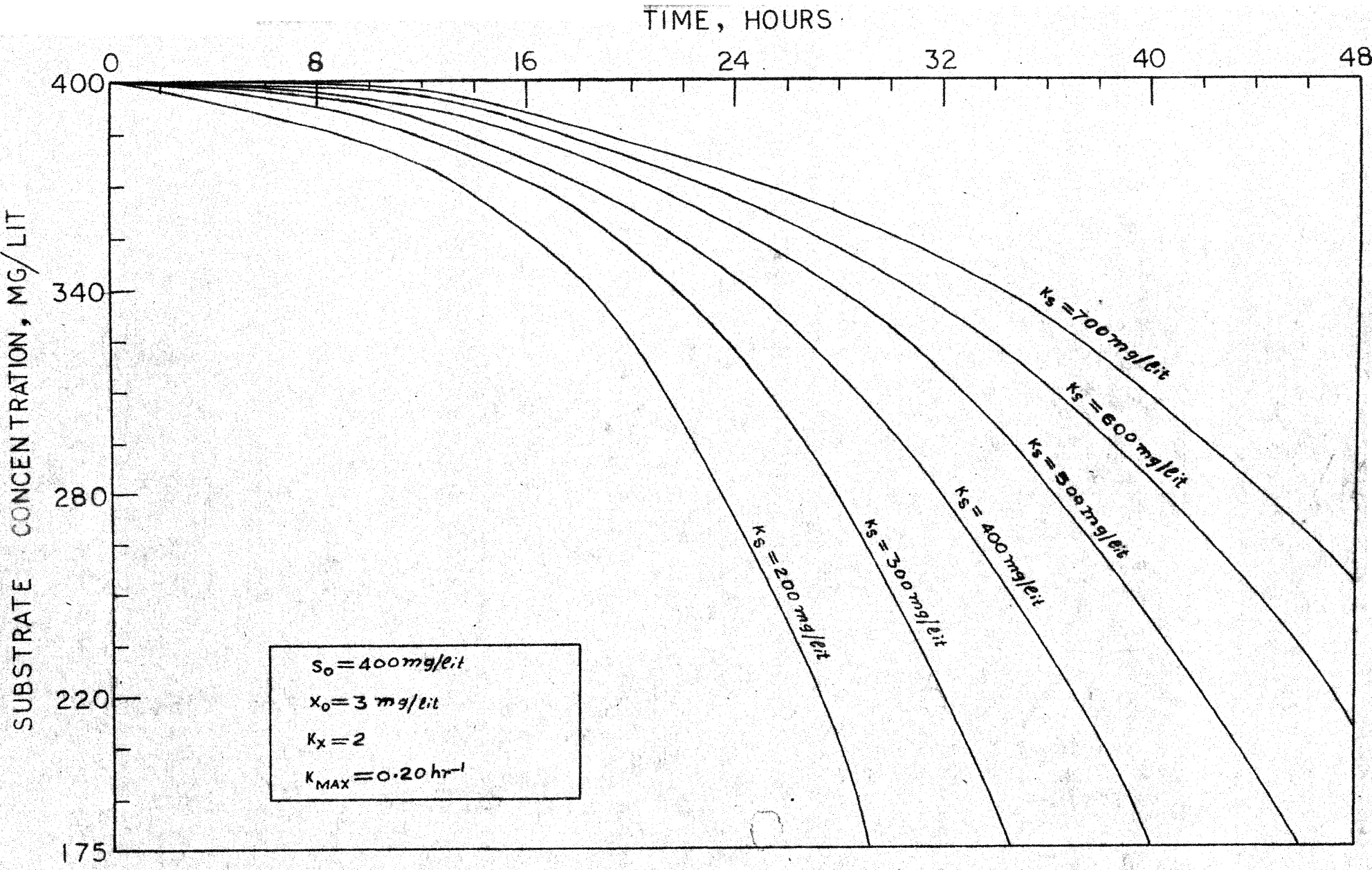
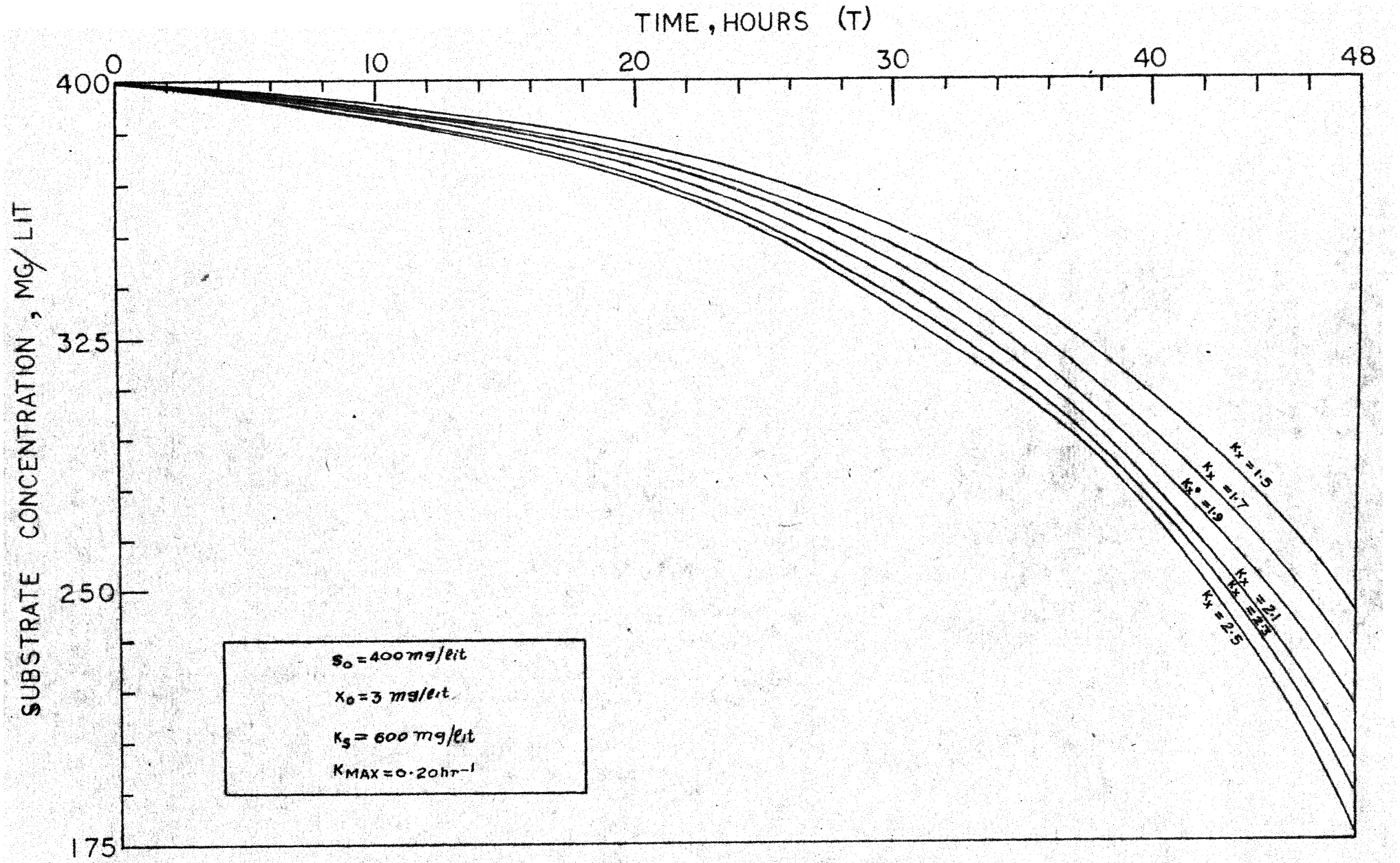


FIG. 8 – THEORETICAL CURVES FOR BOD PROGRESSION [EFFECT OF VARIATION IN K_S]



FIG·9:-THEORETICAL CURVES FOR BOD PROGRESSION [EFFECT OF VARIATION IN K_X]

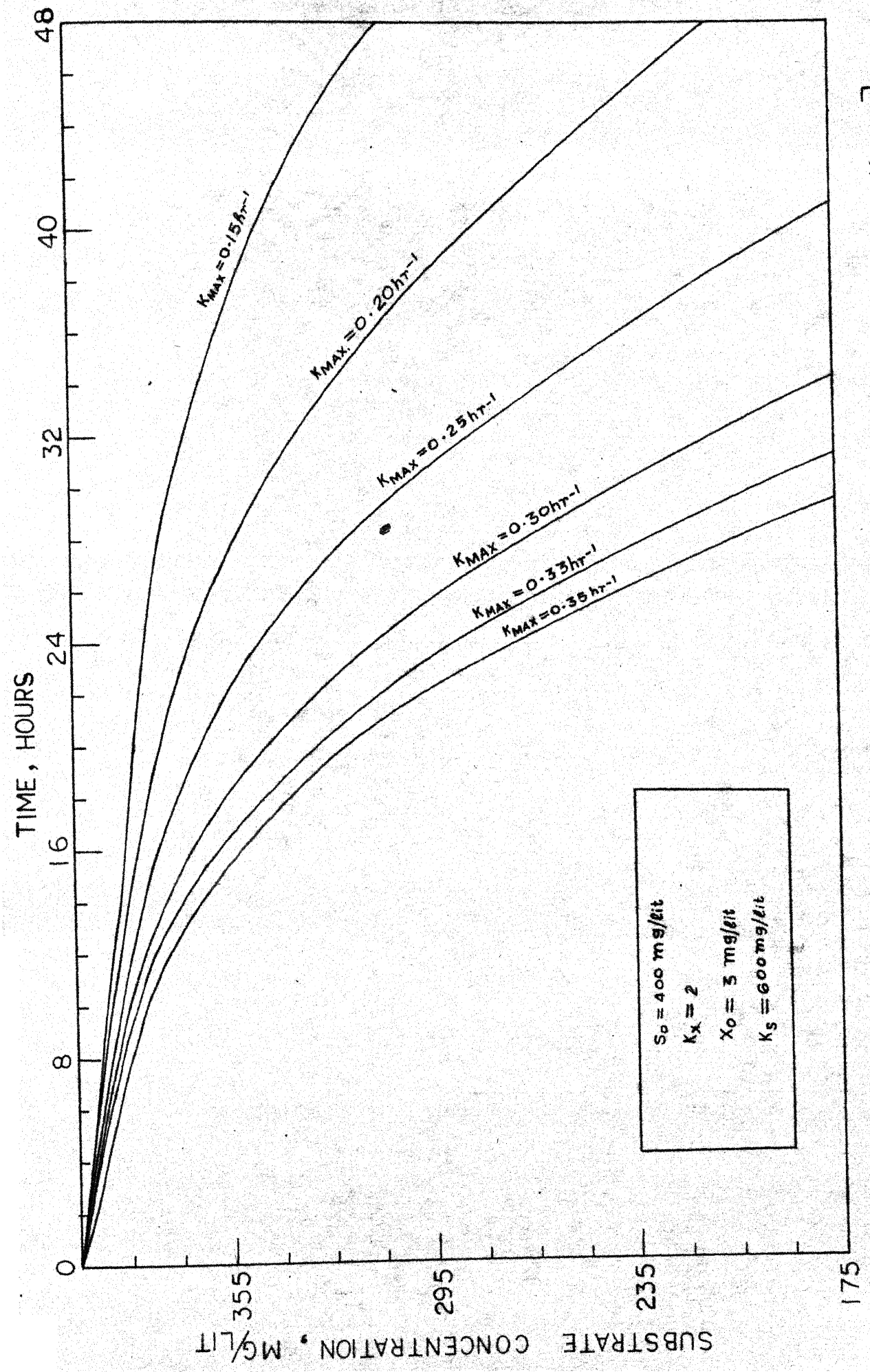


FIG. 10:- THEORETICAL CURVES FOR BOD PROGRESSION [EFFECT OF VARIATION IN $K_{MAX.}$]

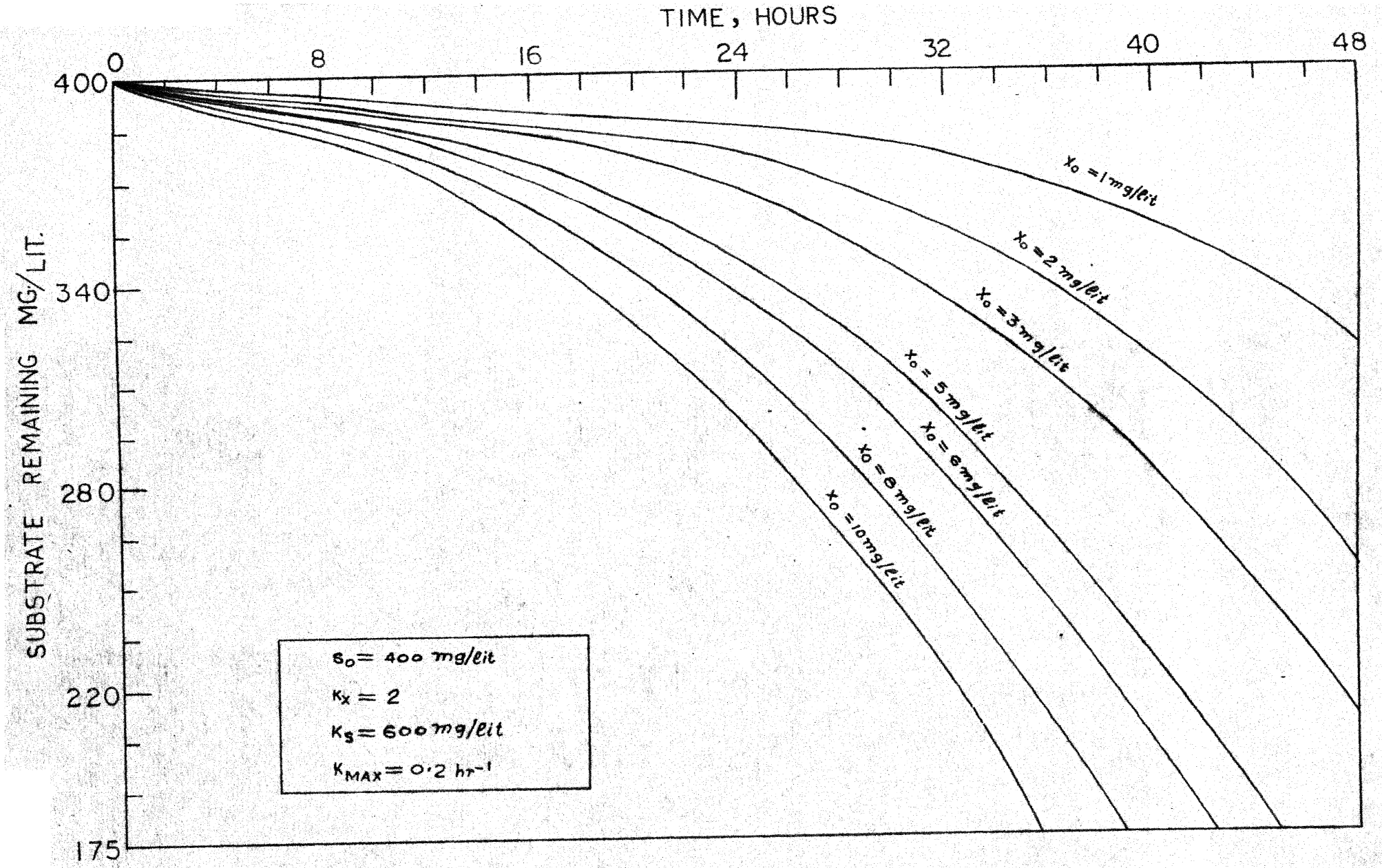


FIG. II:- THEORETICAL CURVES FOR BOD PROGRESSION [EFFECT OF VARIATION IN X_o]

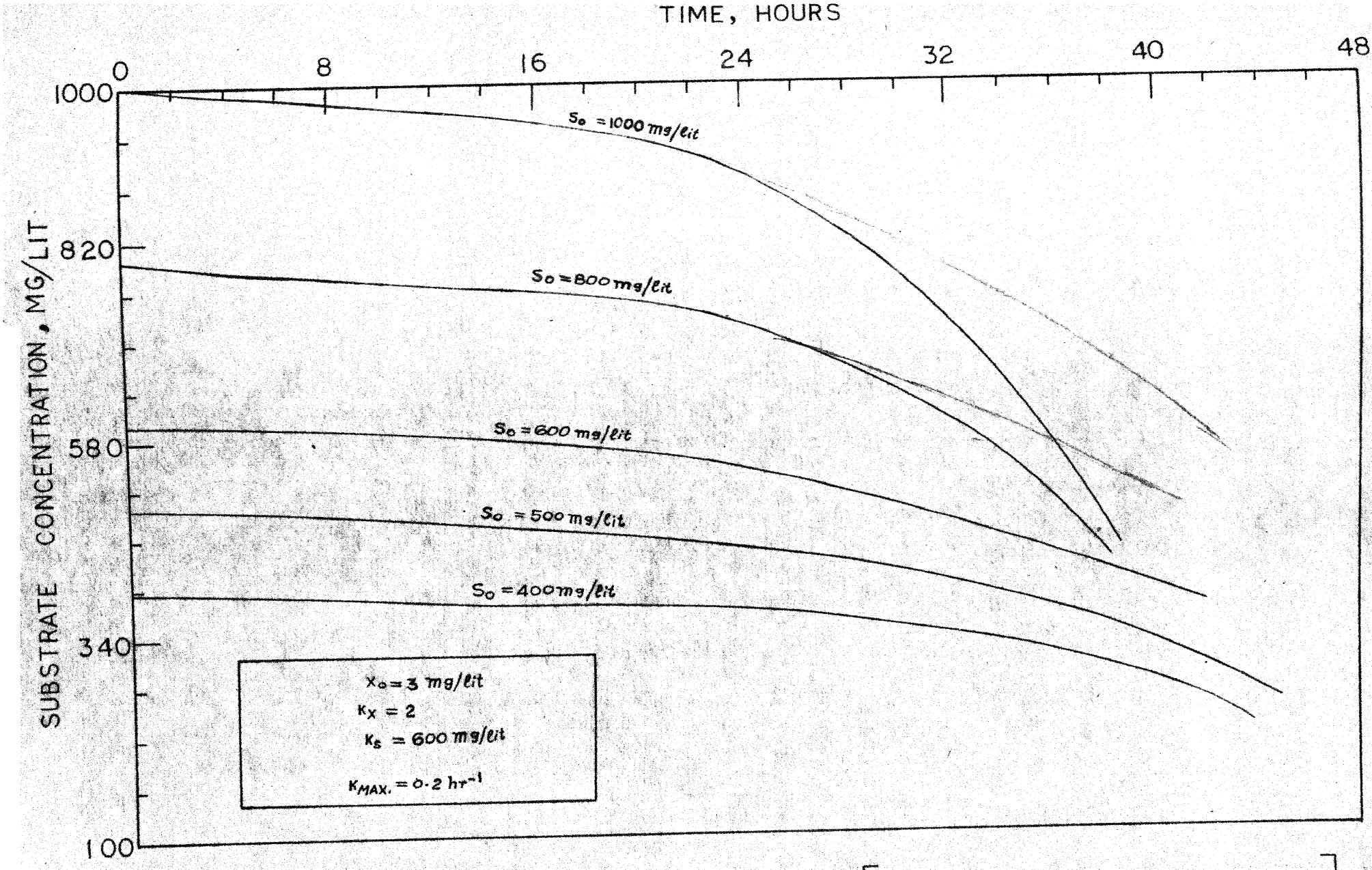


FIG.12:-THEORETICAL CURVES FOR BOD PROGRESSION [EFFECT OF VARIATION IN S_o]

The significance of inclusion of a term representing bacterial concentration in the suggested formulation for the description of kinetics, is clearly borne out by the graphs in Figure 11. Between the arbitrarily chosen range of 1 mg/lit. to 10 mg/lit. for the initial bacterial mass, higher removals of substrate are seen to be associated conceivably with the increased microbial population. Figure 12 reassures the well known fact that the rate of substrate utilization is proportional to concentration of the substrate itself, the concentration raised to some power (the exact evaluation of which requires a rigorous mathematical analysis of the equation (10) reported on page (24). Thus it is convincingly evident that the description of the bio-oxidation kinetics is adequately covered by the proposed rational model and its graphical tracing offers no problem in the compilation of the graphs for various initial conditions, in as much as the digital computer techniques are easily adoptable.

b. Theoretical Curves for Bacterial Growth:

The integrated version of the well known Monod's equation (reported on page 25) provides a useful mathematical tool to study the bacterial growth at different instants of time. The influence and the inter-relationships of the various parameters in the equation are clearly exhibited in figures 13 and 14. Realisation of the fact that bacterial growth is proportional to the substrate utilisation, makes it clear that the effect of variatic in the values of the constants K_{max} and K_S will remain the same

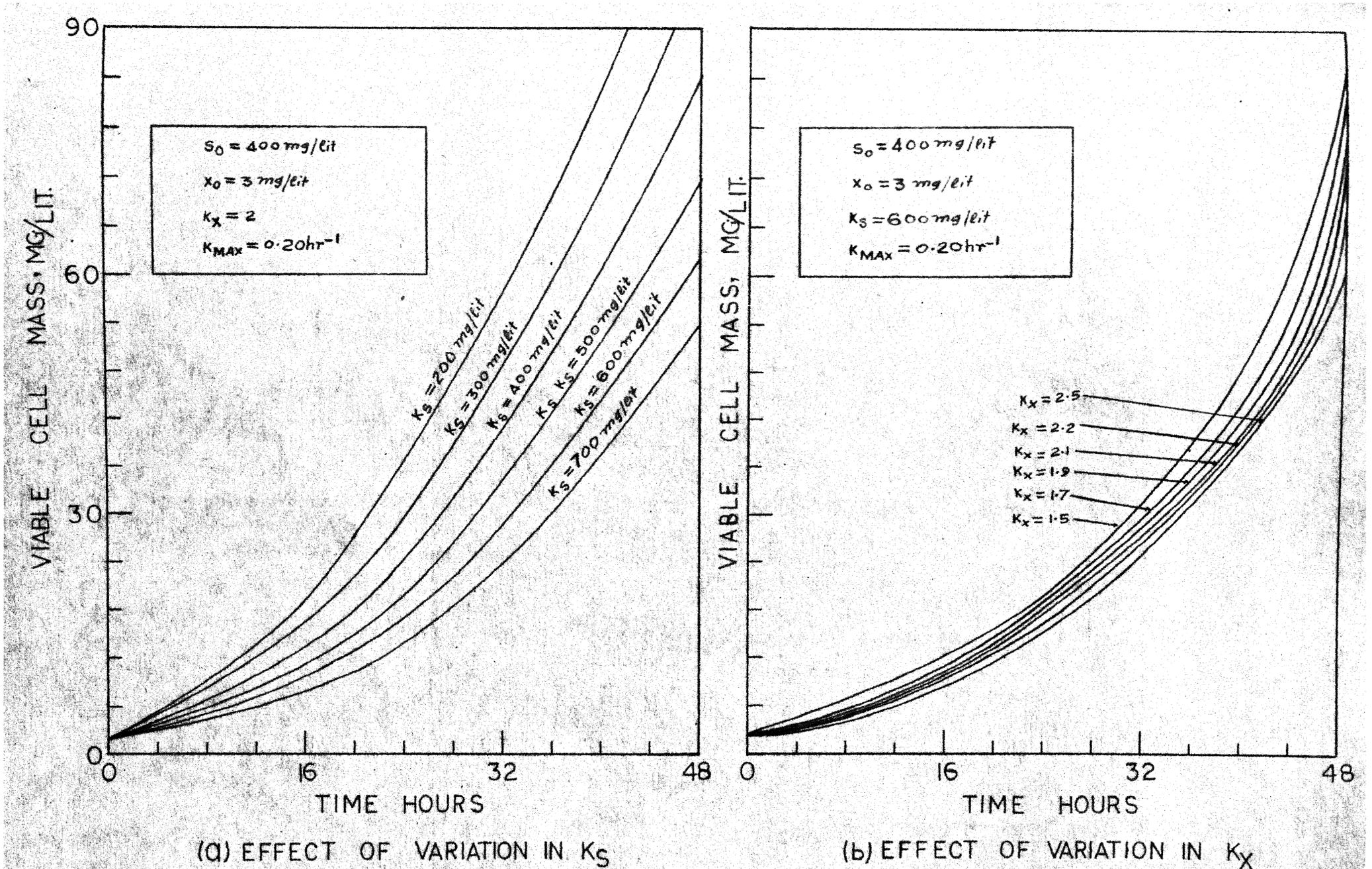
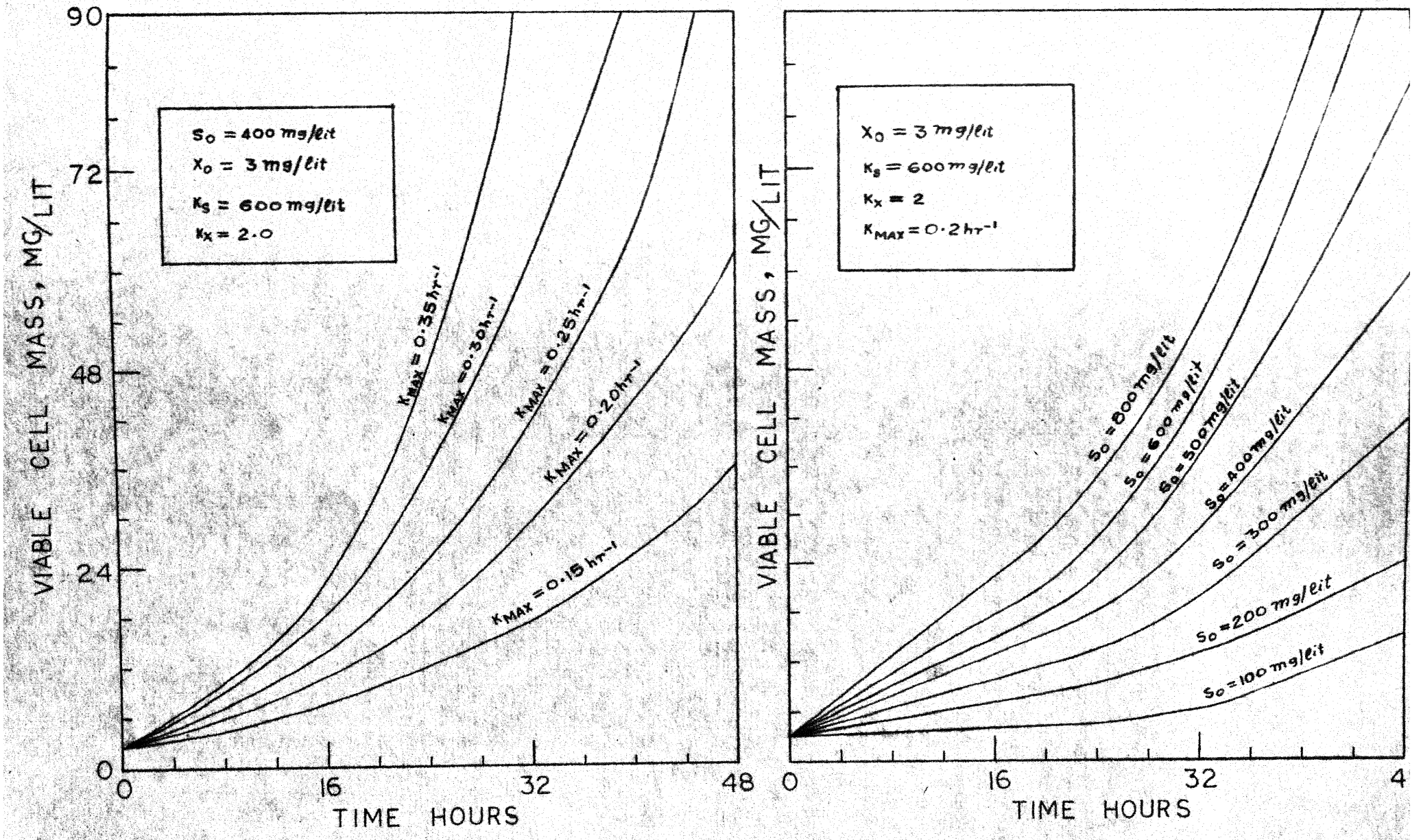


FIG. 13 – THEORETICAL CURVES FOR BACTERIAL GROWTH.



FIG·14:- THEORETICAL CURVES FOR BACTERIAL GROWTH.

for the kinetics of both substrate depletion and bacterial growth under a given environment.

c. Agreement of the Observed Data with the Rational Model:

The experimental studies carried out on the biological oxidation of substrates like Glucose, Peptone and domestic sewage clearly bear evidence to the theory proposed. The observations indicate that the rate of substrate utilization by the microbial flora depends not only on the instantaneous substrate concentration as a monomolecular kinetics would suggest, but also on the instantaneous concentration of the micro-organisms. It is a well known fact that the ultimate foundations underlying the biochemical oxygen demand of waste materials are the enzyme catalysed processes involved in the growth and multiplication of the organisms acting on these materials. In view of the significant role played by bacteria in the removal of substrate, caution should be exercised in the selection of proper type of seed and its amount while performing the BOD tests by the standard delution technique.

Table II shows the effect of the different seed to substrate ratio on the BOD progression of substrates viz., Glucose, Peptone and domestic Sewage. It can be noted that for a given initial seed, the rate of substrate depletion increases with the corresponding increase in the substrate concentration. The choice of domestic seed in the experiment was made in particular because of its common presence in the polluted rivers, for which the rational model was proposed. This has also given the opportunity to study in details the ability of the complex microbial flora present in

the seed in consuming simple (glucose) as well as complex (sewage) substrate. From Table II it is also observed that the rate of Glucose utilization remains more or the less the same as that of sewage. This finding goes to substantiate the conclusions arrived at by investigators like Garret, Sawyer andGGaudy that the kinetics of the removal of complex substrates follows the same relationship that are applicable to the utilization of simple substrates.

TABLE II

Substrate	Initial concentration of substrate (on BOD in Mg/l.	Seed to substrate ratio	BOD exerted out the end of 48 hrs. as a % ge of the theoretical BOD
Glucose	412	1/160	66
		1/80	73
		1/40	81
	262	1/96	39
		1/48	55
		1/24	62
	131	1/48	21
		1/24	36
		1/12	54
Peptone	460	1/160	61
		1/80	66
		1/40	71
	272	1/96	55
		1/48	58
		1/24	61
	138	1/48	27
		1/24	39
		1/12	56
Domestic sewage	182	1/72	41
		1/36	60
		1/18	79

* A - Ratio of initial concentration of seed to initial substrate concentration.

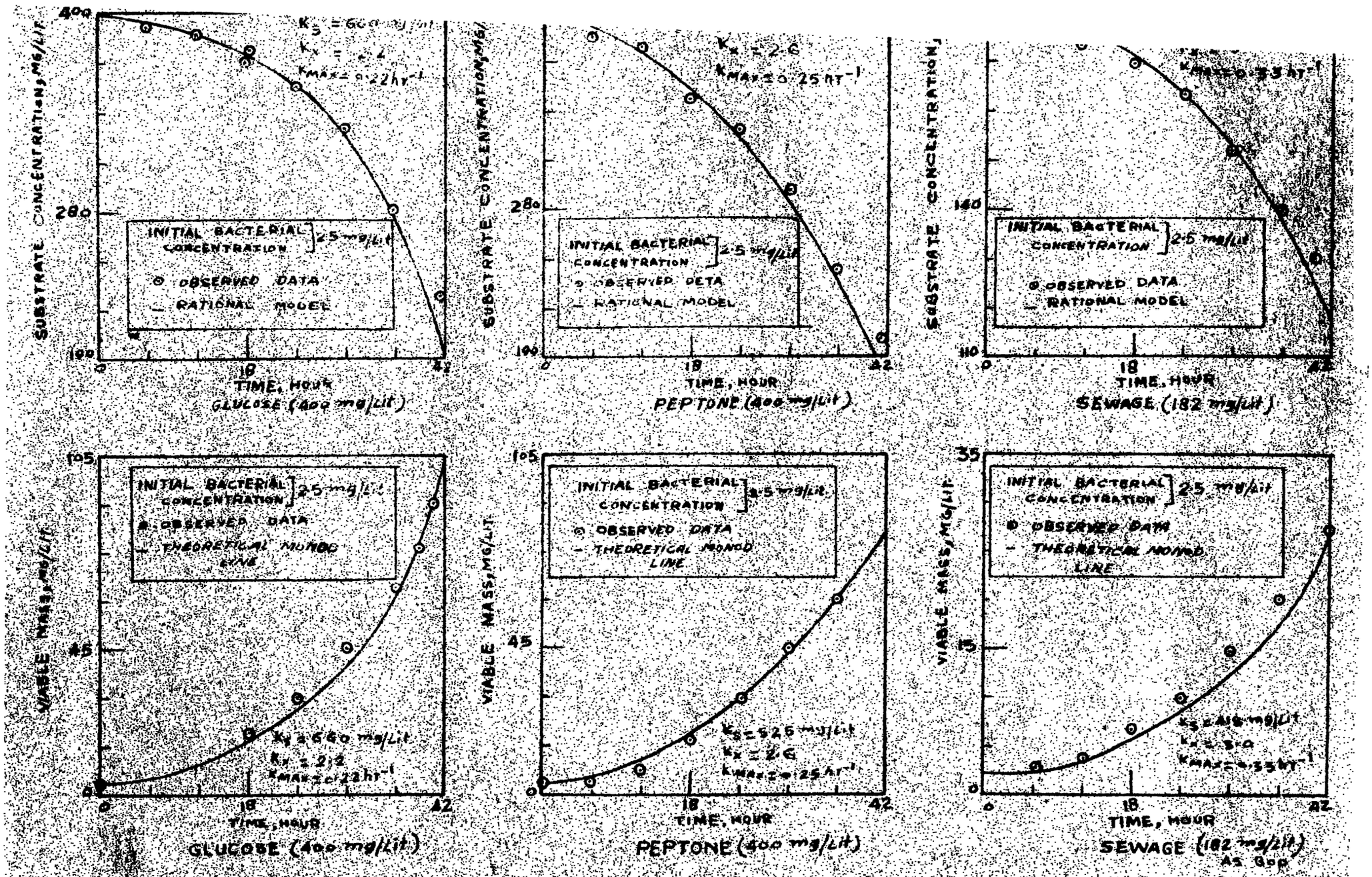


FIG. 15 :- AGREEMENT OF THE OBSERVED DATA WITH THE THEORETICAL DATA.

NOTE :- THE THEORETICAL LINES FOR SUBSTRATE-DEPLETION AND BACTERIAL GROWTH HAVE BEEN DRAWN WITH THE VALUES OF THE CONSTANTS K_x, K_s AND K_{MAX} OBTAINED GRAPHICALLY FROM THE OBSERVED DATA AS SHOWN IN FIG. 19.

Figure 15 brings out in essence the striking resemblence of the observed data to the postulated model. The graphs shown in the figure correspond to the kinetics of substrate utilization and bacterial growth for the three substrates, acted upon by identical bacterial mass. Only 3 sets of readings are recorded in the graphs corresponding to the initial substrate concentrations of 400 mg/lit. for Glucose, 400 mg/lit. for Peptone and 182 mg/lit. for domestic sewage. The initial bacterial concentration adopted was 2.5 mg/lit. The values of the constants (K_X, K_S and K_{Max}) in the theoretical calculations for BOD exersion and Bacterial growth were taken from the graphical plot made in fig. 19 for the experimental observations. It is very clear that the observations for BOD exertion fit closely to the proposed model and those for bacterial growth, to the Monod equation.

Referring back to Fig. 1 to 7, showing the experimental observations, the presence of a lag period can be noticed, especially at lower concentration of the seed, in the early portion of the bacterial growth curves after which they rise concave upward. The rational model presented by the author does not account for this type of kinetics with a lag period. The presence of the lag period is attributable to the inability of the reduced microbial population in the initial stages to consume the substrate.

d. Comparison with the Streeter-Phelps Formulation:

With the distinctive features of the proposed model well recognized, one can strike a comparison between the two formulations -- the rational and the first order, primarily with a view to pinpoint the inadequacy of the later equation in des-

cribing the substrate depletion kinetics. First of all, the application of the monomolecular equation is empirical in nature and the ultimate demand is entirely theoretical. Neither of the two constants* (K' and L) that appear in the equation can be determined directly. They call for an extensive application of 'the curve fitting techniques such as those suggested by Theriault[32], Thomas[3] and others. L can not be determined experimentally because it is BOD at infinite time. Thus K' and L as ordinarily calculated serve only as statistical constants to shape the BOD curve instead of acting as physical and biological parameters.

The application of the proposed model, on the otherhand, is rational because none of the constants in the formulation is hypothetical. K_X stands for the degree of conversion of the substrate into the protoplasmic mass while K_S and K_{max} go to describe adequately the bacterial kinetics. The initial conditions are well recorded in the presence of the parameters X_o and S_o. The formulation attempts fairly satisfactorily in the correlation of the BOD removal kinetics with the enzymes and growth kinetics in bacteria.

Figures 16, 17 and 18 clearly record the comparison of the two formulations. In the early stages of BOD exertion the first order equation stipulates a higher percentage of BOD consumed

---

* The constants are the rate constant K', and the ultimate oxygen demand L, which appear in the Streeter-Phelps equation,

$$Y = L\,(1-e^{-K't})$$ where, Y - BOD exerted in time t.

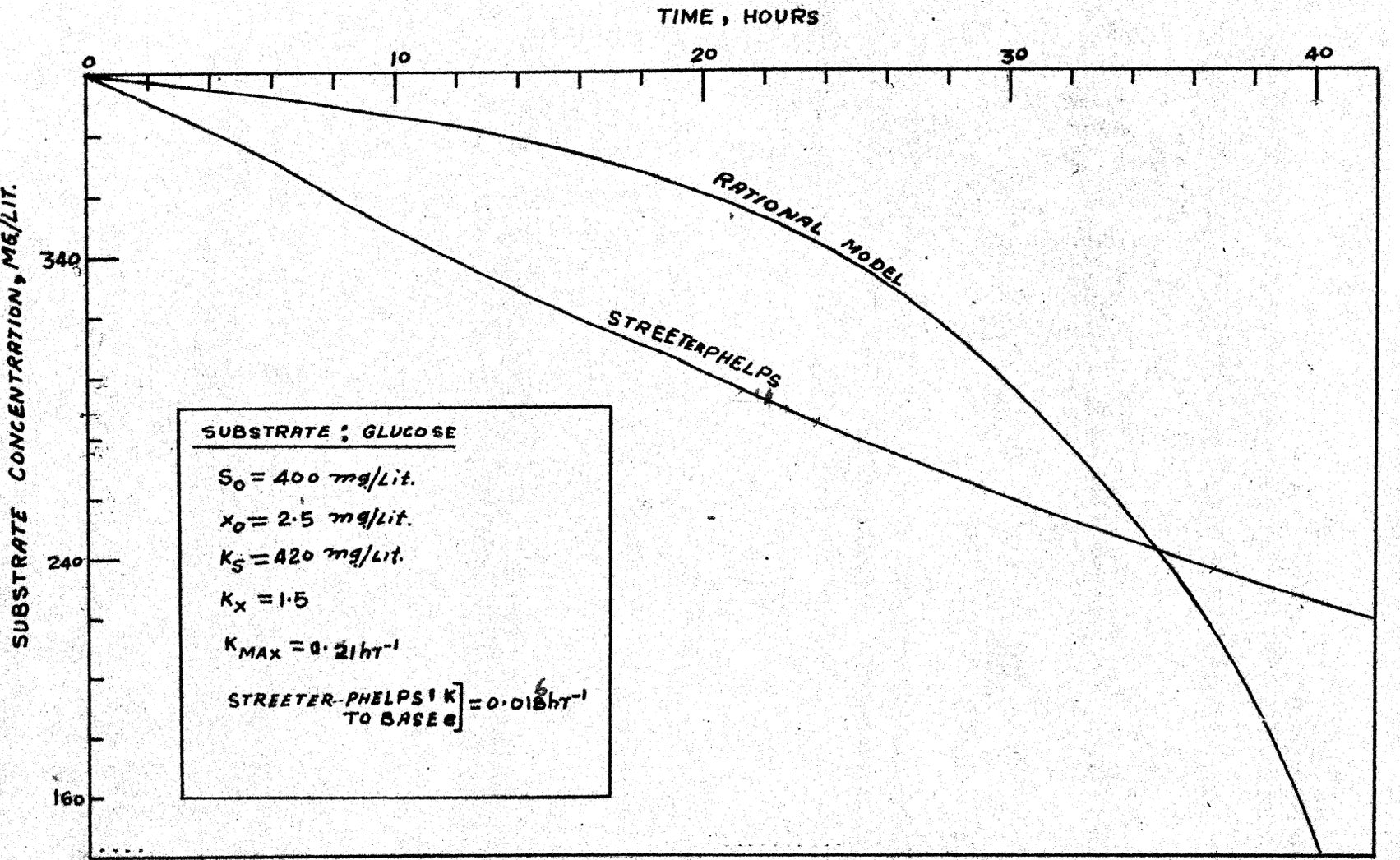


FIG. 16 COMPARISON OF STREETER–PHELPS AND RATIONAL MODEL.

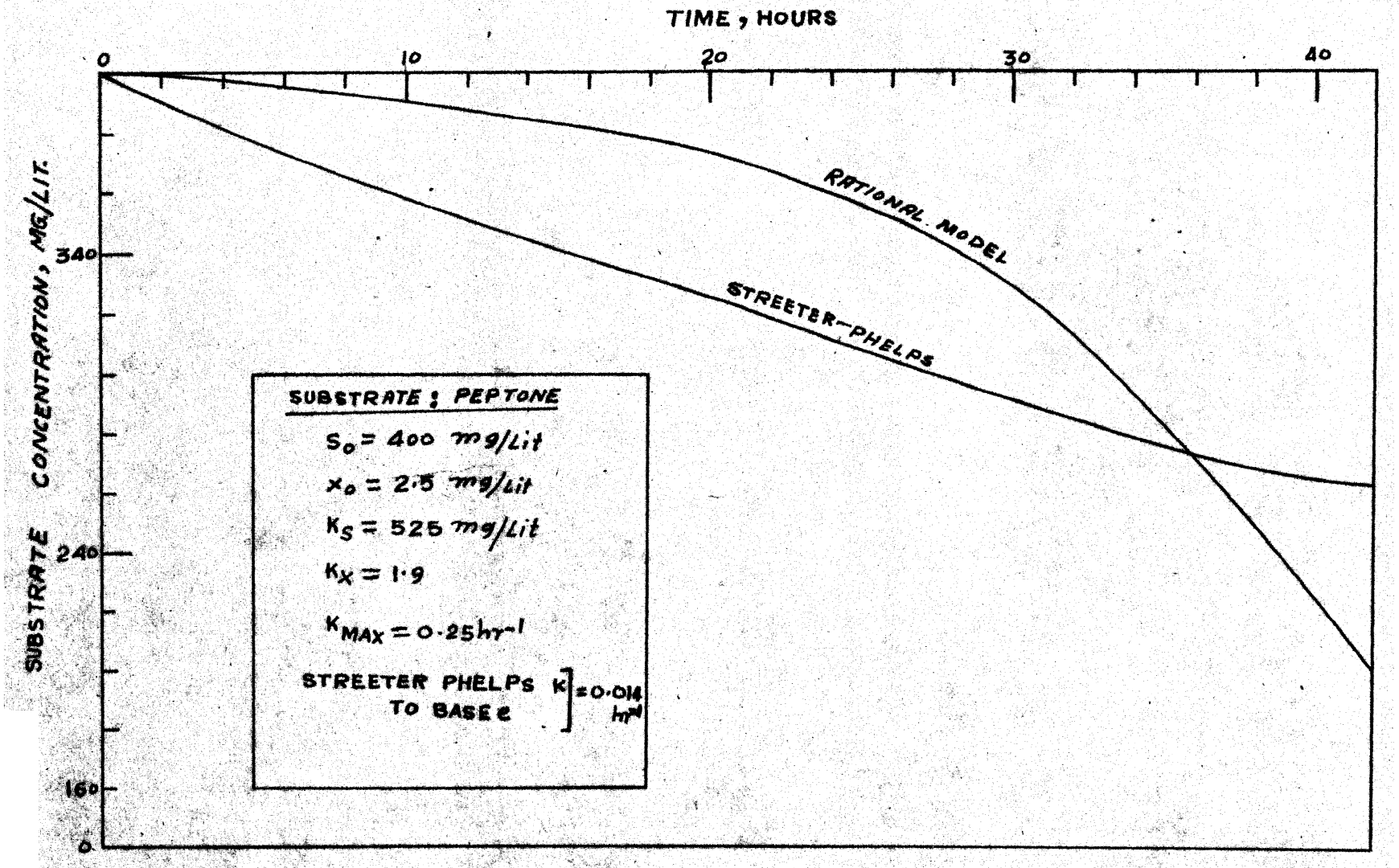
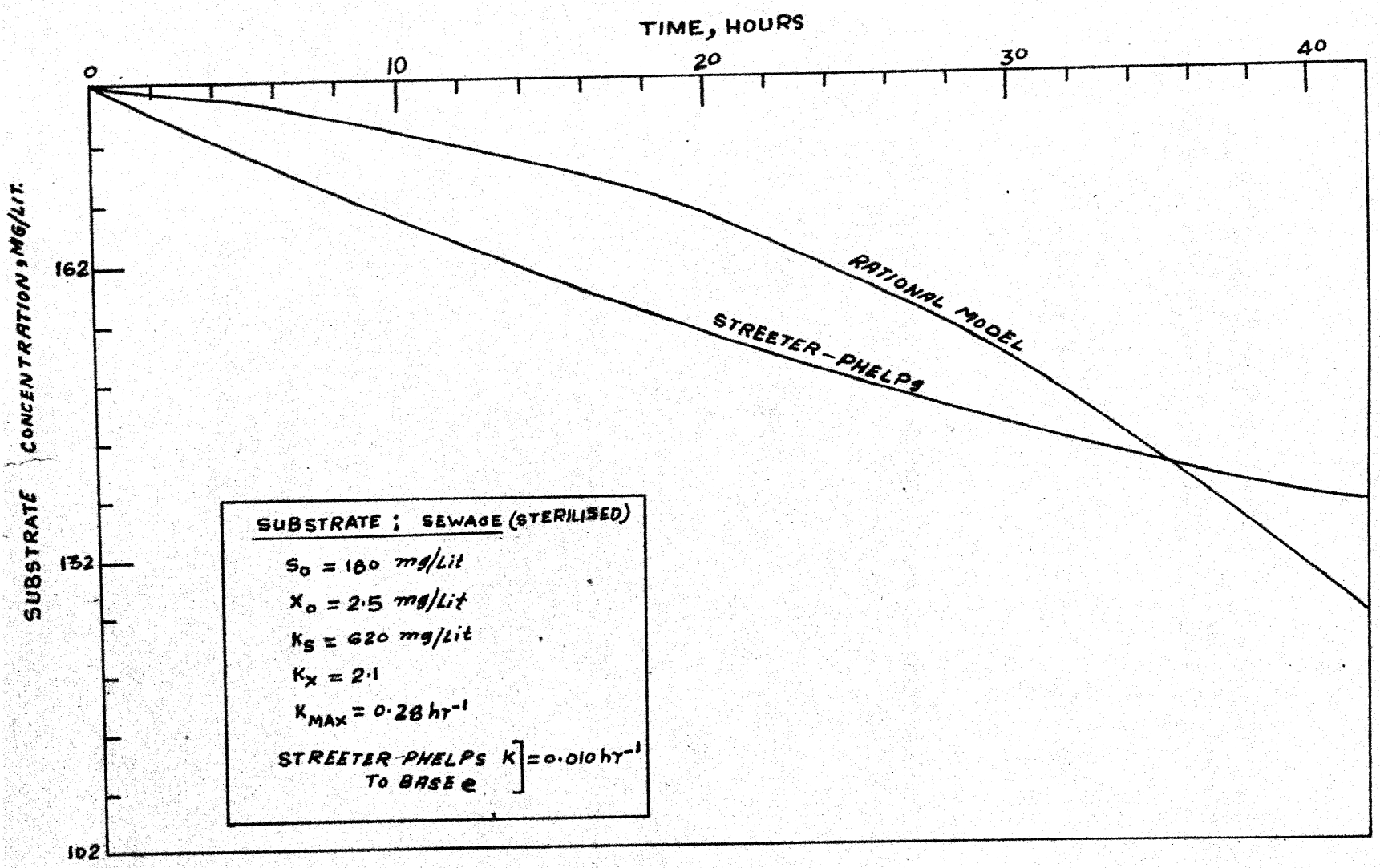


FIG. 17 :- COMPARISON OF STREETER- PHELPS AND RATIONAL MODEL



Fig·18:- COMPARISON OF STREETER-PHELPS AND RATIONAL MODEL

than what is postulated by the rational equation. The values of the constants K_s, K_x and K_{max} were chosen arbitrarily and the graphs, traced for the computed values in accordance with the mathematical model. The fitting of the Streeter-Phelps curves was done by least-squares method. It is noticed that while these curves tend to remain asymptotic to the time-axis with increasing time, the rational ones droop down considerably. These graphs thus provide sufficient insight into the descriptions of kinetics of substrate depletion and one can obviously conclude that the first order equation is highly unjustified in the light of the existing knowledge on the kinetics of BOD reactions.

e. Determination of the Constants:

A major task in the verification of the presented model with the observed data lies in the evaluation of the constants K_x, K_s and K_{max}. The value of K_x, as far as theory goes, should remain constant for the same substrate and seed conditions. The reported values in Table IV however contradict this and the fluctuations in the values of K_x are mainly attributable to the changes in behaviour of the environment characterised by the hetrogeneous microbial population. K_x varies from 1.1 to 3.7 for Glucose, from 1.7 to 3.2 for Peptone and from 2.4 to 3.7 for domestic sewage.

TABLE III

VALUES OF K_X FROM OBSERVED DATA

Substrate	Initial concentration of substrate (mg/l)	Seed to substrate ratio (R)	Values of K_X
Glucose	400	1/160	2.3
		1/80	1.6
		1/40	3.7
	240	1/96	1.8
		1/48	2.5
		1/24	2.7
	120	1/48	1.1
		1/24	1.7
		1/12	2.2
Peptone	400	1/160	1.7
		1/80	3.1
		1/40	4.0
	240	1/96	3.2
		1/48	2.8
		1/24	2.7
	120	1/48	1.8
		1/24	1.7
		1/12	2.4
Domestic Sewage	182	1/72	2.4
		1/36	2.9
		1/18	3.7

The evaluation of the Kinetic constants K_X and K_{max} is facilitated by the linear plot of the Monod's equation as suggested by Lineweaver and Bert. $U = \frac{U_{max} \; X \; S}{K_S + S}$

Taking reciprocals on both sides of the above equation,

$$\frac{1}{u} = \frac{K_S}{K_{max}} \left(\frac{1}{S}\right) + \frac{1}{K_{max}}$$

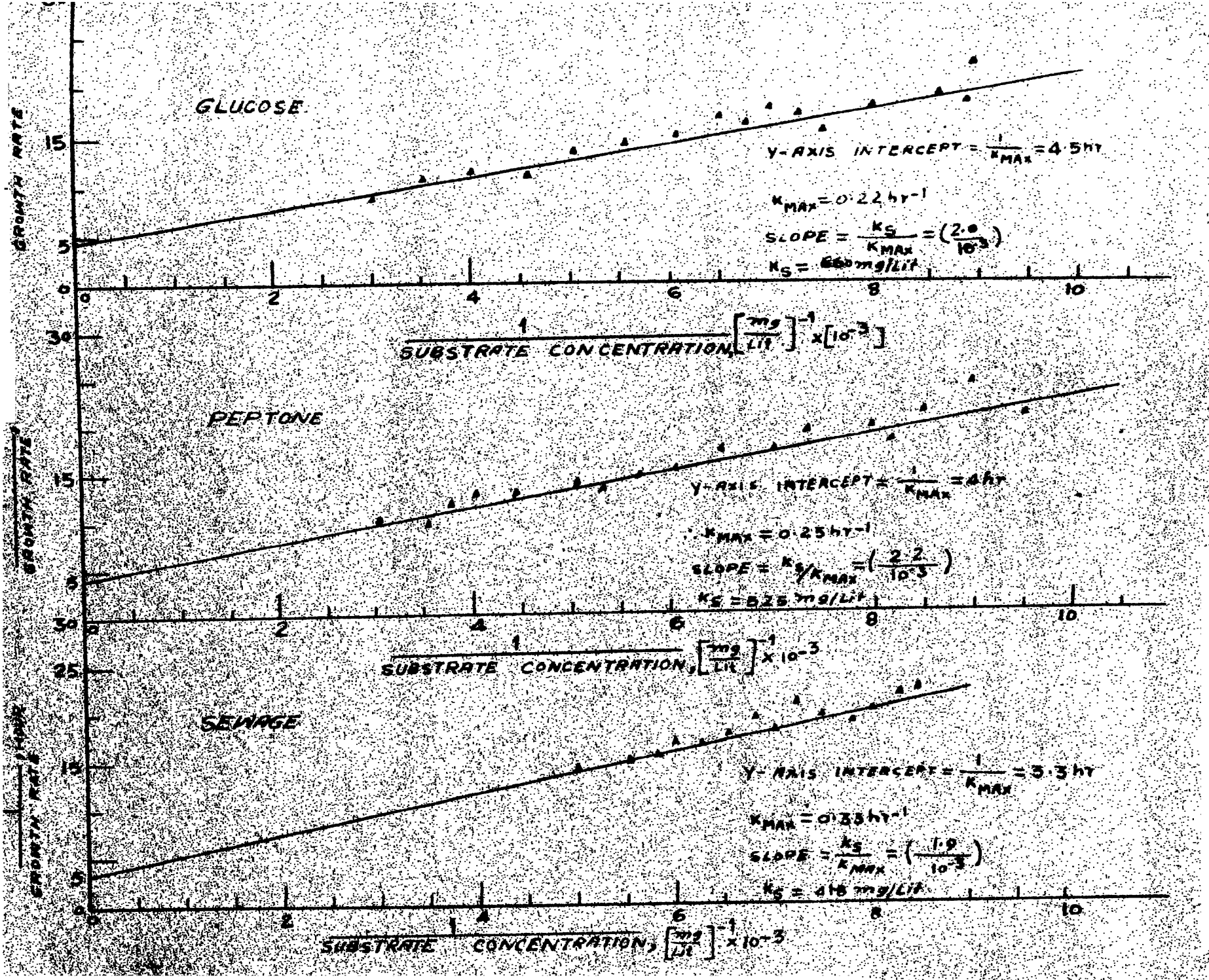
It can be seen that by plotting 1/u vs. 1/S, a straight line of slope K_S/K_{max} and intercept $1/K_{max}$ should result, if the Michaelis equation holds. + Figure 19 shows such a plot for the constants, K_S and K_{max}.

TABLE IV

VALUES OF K_S AND K_{max} FROM THE OBSERVED DATA

Substrate	K_S (mg/lit.)	K_{max} (hr^{-1})
Glucose	660	0.22
Peptone	525	0.25
Sewage	418	0.33

The major revelations of this table are to the values of K_{max} increases as the substrate is more and more complex (2) the values of K_S decreases with increasing complexity of the substrate, which should be expected in the light of arguments under the subheading (a) in this chapter. It was assumed that the endogeneous respiration will be negligible in the Warburg - Flasks for the time of duration of the experiment (48 hours). To account for the endogeneous uptake


GLUCOSE
GROWTH RATE
15
5
0
0
2
4
6
8
10
1
SUBSTRATE CONCENTRATION, $\left[\frac{mg}{Lit}\right]^{-1} \times [10^{-3}]$
y-AXIS INTERCEPT = $\frac{1}{K_{MAX}}$ = 4.5 hr
$K_{MAX} = 0.22\ hr^{-1}$
SLOPE = $\frac{K_S}{K_{MAX}} = \left(\frac{2.0}{10^{-3}}\right)$
K_S = 660 mg/Lit
PEPTONE
GROWTH RATE
30
25
15
5
0
0
2
4
6
8
10
1
SUBSTRATE CONCENTRATION, $\left[\frac{mg}{Lit}\right]^{-1} \times 10^{-3}$
y-AXIS INTERCEPT = $\frac{1}{K_{MAX}}$ = 4 hr
$\therefore K_{MAX} = 0.25\ hr^{-1}$
SLOPE = $K_S/K_{MAX} = \left(\frac{2.2}{10^{-3}}\right)$
K_S = 525 mg/Lit
SEWAGE
GROWTH RATE / HOUR
25
15
5
0
0
2
4
6
8
10
SUBSTRATE CONCENTRATION, $\left[\frac{mg}{Lit}\right]^{-1} \times 10^{-3}$
y-AXIS INTERCEPT = $\frac{1}{K_{MAX}}$ = 3.3 hr
$K_{MAX} = 0.33\ hr^{-1}$
SLOPE = $\frac{K_S}{K_{MAX}} = \left(\frac{1.9}{10^{-3}}\right)$
K_S = 418 mg/Lit

mentioned equation was modified by Tench and Morton [31] as follows:

$$\frac{1}{\mu - \mu_o} = \frac{K_S}{(K_{max} - \mu_o)} \left(\frac{1}{S} \right) + \frac{1}{(K_{max} - \mu_o)}$$

where, μ_o is the respiration rate with no added substrate.

Careful observation shows that there need not be any conflict over the discrepancies in the value of K_S and K_{max} reported by early investigators. Tench and Morton have obtained the values of K_S for Glucose and Peptone as 133 and 296 mg/lit. respectively. It should be borne in mind that they used in their experiments the activated - sludge-seed which harbours some organisms quite uncommonly noticed in the domestic sewage seed. (e.g. Sphaerotilus natans). Since the author has adopted small initial concentrations of the micro-organisms as contrasted to the mass culture work published by many research-workers, there is bound to be some inconsistency in the reported values of K_S and K_{max}. Garret and Sawyer [13] have indicated through their experiments, the values of K_{max} as 0.18/hr and 0.21/hr respectively for glucose and peptone at 20°C. They postulated that only two phases -- a log phase and a transition to the stationary phase -- were of practical importance in the definition of the reaction kinetics of the aerobic biological processes. They objected Monod's theory on the basis of their experimental results and suggested that 'the equation denies the existence of a constant rate of growth above critical concentrations of food, although this is the most frequently observed phenomenon related to the growth of bacteria'. However, experimental data from

Wilson's experiments were found to fit close to the Monod's equation rather than to the two-phase formulation. Several cycles of experiments are needed supporting the validity of the two theories. The author's own experiments have shown that Monod's equation provides a striking fit to the observed data.

i) Practical Applications:

The significance of the rational model lies in its direct application to the field conditions. It can be very easily adopted by the agencies concerned in the water-pollution-abatement programmes . Its amenability for solution by modern digital computer techniques should provide adequate attraction especially to those with whom lies the responsibility of the data collection in river-pollution studies. Just the different sets of BOD-graphs need to be compiled and thereafter it remains to match the observed profiles of pollution with the theoretical graphs.

CHAPTER VII

CONCLUSIONS

A deeper understanding of the kinetics involved in the substrate depletion is essential for adoption of methods to predict the exact degree of organic pollution in streams. The author has drawn the following conclusions based on his experimental works.

1. The rate of progression of BOD in streams is found to be proportional not only to the concentration of the remaining B.O.D. at that instant but also to the bacterial concentration. The experimental observations validate the proposed theory for the BOD kinetics based on a realistic and rational approach.

2. The bacterial growth observed in the experiments follows the curve typified by Monod's equation. The two phase formulations of Garret and Sawyer do not hold good in particular for the author's observations. No existence of the 'plateau' reported by Busch et al was found in the geometry of the BOD curves.

3. The rational model has several advantages over the first order formulation. First of all, its application for practical problems is quite simple and direct. The extensive use of the curve fitting techniques required for the first-order-reaction - kinetics is done away with. All that one has to do is to match the experimentally observed curves with the theoretical graphs

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GLOSSARY OF SYMBOLS

K' = The rate constant in the first-order BOD equation

K_m = Michaelis-Menten Constant

K_{max} = Maximum Growth Rate of the Bacterial Cells

K_S = Saturation Constant

K_X = Reciprocal Constant of the Yield Coefficient

L = Ultimate oxygen demand in the first-order BOD equation

S = Substrate concentration

T = Time

μ = Growth rate of bacterial cells

X = Bacterial concentration or Viable Mass expressed in concentration units.

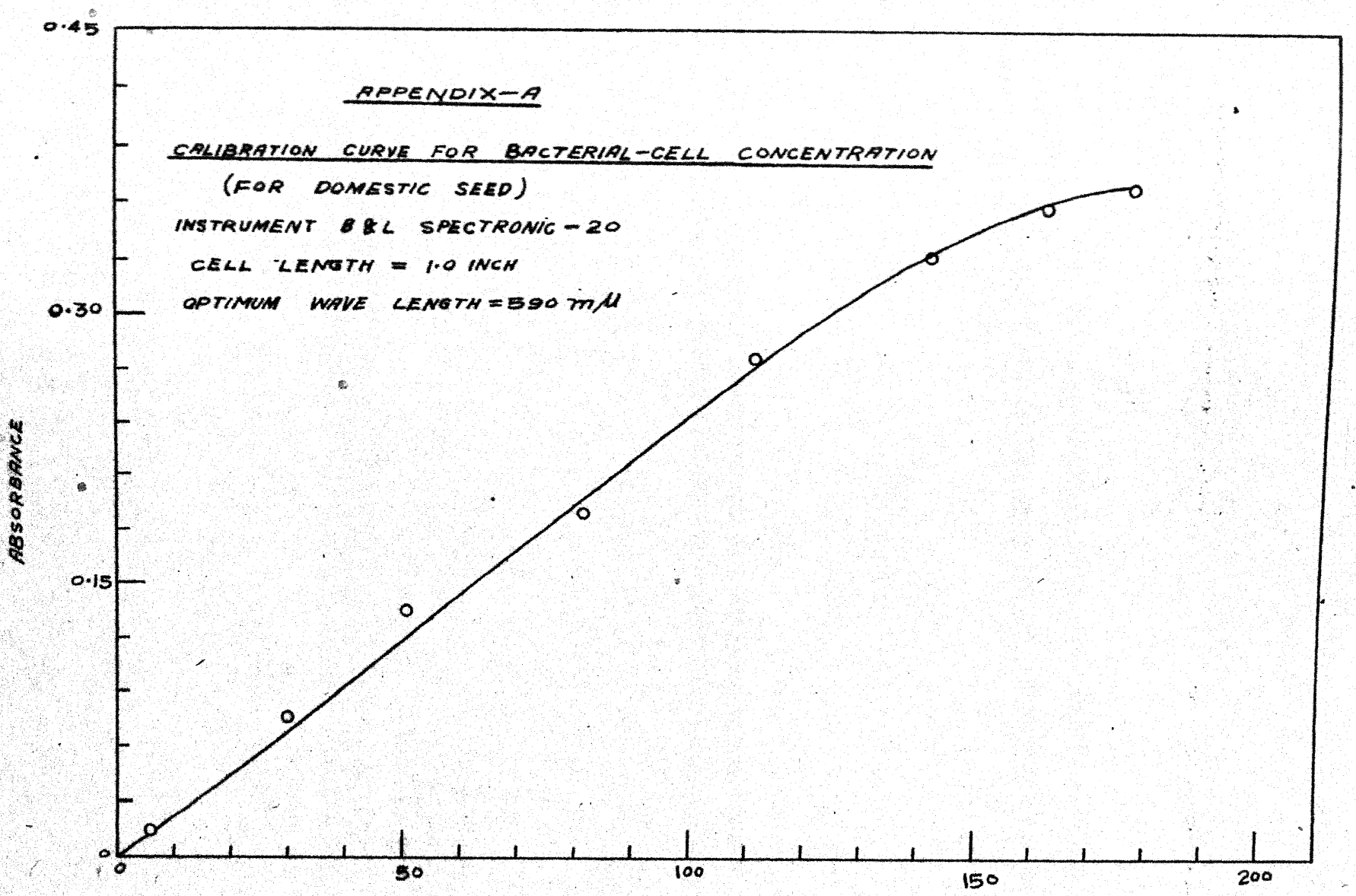
APPENDIX-A
CALIBRATION CURVE FOR BACTERIAL-CELL CONCENTRATION
(FOR DOMESTIC SEED)
INSTRUMENT B & L SPECTRONIC - 20
CELL LENGTH = 1.0 INCH
OPTIMUM WAVE LENGTH = 590 mμ
ABSORBANCE
0.45
0.30
0.15
0
0
50
100
150
200
VIABLE CELL CONCENTRATION, MG./Lit.

FIG. 20

APPENDIX - B-I

SUBSTRATE: GLUCOSE
CONCENTRATION: 400 mg/lit
COD: 412 mg/lit.

TABLE OF OBSERVATIONS

Time in Hours	* BOD EXERTED in mg/lit			** VIABLE CELL MASS IN mg/lit		
	R = 1/8	R=1/40	R=1/20	R=1/160	R=1/80	R=1/20
4	2	3	7	3	8	12
8	6	10	30	7	11	16
12	7	20	38	9	20	23
16	20	34	60	14	21	32
20	21	42	86	20	32	35
24	44	70	126	29	40	46
28	60	108	190	34	48	60
32	84	141	238	54	59	62
36	121	212	281	63	75	67
40	150	252	302	80	89	75
44	252	284	320	93	102	86
48	273	302	331	118	110	91

**Only the final observations (i.e. after reading from the calibration graph) are given.
** The manometric readings have been converted into the unit of BOD mg/lit.

APPENDIX - B-II

TABLES OF OBSERVATIONS

SUBSTRATE: GLUCOSE
CONCENTRATION = 240 mg/lit.
COD = 262 mg/lit.

Time in Hours	BOD EXERTED IN mg/lit			VIABLE CELL MASS in mg/lit		
	R = 1/96	R=1/48	R=1/24	R=1/96	R = 1/48	R=1/24
4	II	2	8	2.6	6.4	12.8
8	2	7	12	5.6	9.2	14.1
12	7	11	20	6.4	10.4	18.0
161	9	20	29	10.0	14.8	22.8
20	11	25	43	13.2	17.4	25.2
24	15	33	63	14.0	23.8	30.0
28	28	47	79	18.1	26.8	33.8
32	37	67	100	24.8	30.8	40.0
36	46	84	116	29.2	41.2	44.0
40	65	103	143	30.0	46.0	48.2
44	76	129	154	43.6	52.0	54.0
48	101	144	162	56.1	58.1	60.8

APPENDIX - B-III

TABLE OF OBSERVATIONS

SUBSTRATE: GLUCOSE
CONCENTRATION: 120 mg/lit
COD: 131 mg/lit.

Time in Hours	BOD EXERTED IN mg/lit.			VIABLE CELL MOSS IN mg/lit		
	R=1/8	R=1/4	R=1/2	R=1/8	R=1/4	R=1/2
4	=	1.0	2.5	3.2	5.8	11.1
8	1.0	3.0	6.4	4.0	7.9	13.1
12	2.5	5.5	10.2	5.2	8.0	13.8
16	2.7	7.5	16.6	5.6	10.1	16.7
20	5.5	12.6	21.5	7.2	11.9	18.1
24	7.0	13.5	25.6	7.4	13.2	20.4
28	7.5	19.0	35.0	9.8	16.0	22.4
32	12.5	21.5	48.8	11.0	17.4	25.1
36	14.0	25.5	47.5	13.6	20.4	27.6
40	20.0	31.6	56.2	16.0	22.2	30.0
44	24.0	38.0	62.5	18.0	25.8	31.8
48	27.5	47.5	71.5	20.6	28.0	32.9

APPENDIX - B-IV

TABLE OF OBSERVATIONS

SUBSTRATE = PEPTONE
CONCENTRATION = 400 mg/lit
COD = 460 mg/lit.

Time in Hours	BOD EXERTED IN mg/lit			VIABLE-CELL MASS IN mg/lit		
	R =1/160	R=1/80	R=1/20	R=1/160	R=1/80	R=1/20
4	4	6	12	3	8	15
8	6	15	30	7	14	21
12	14	30	52	8	16	24
16	27	46	84	13	24	28
20	40	66	120	21	30	33
24	61	106	176	27	41	38
28	90	158	220	39	48	43
32	131	201	268	53	60	48
36	170	250	290	64	67	54
40	276	276	301	78	78	65
44	259	290	316	94	87	78
48	282	307	324	110	96	85

APPENDIX - B - V

TABLE OF OBSERVATIONS

GLUCOSE: 240 mg/lit.
CONCENTRATION: 240 mg/lit.
COD. 272 mg/lit.

TIME IN HOURS	BOD EXERTED IN mg/lit.			VIABLE CELL MASS IN mg/lit.		
	R = 1/96	R=1/48	R=1/24	R=1/96	R=1/48	R=1/24
4	1	2	7	4.0	6	12
8	4	10	17	4.0	9	14
12	5	19	31	8	11	18
16	13	30	41	9	14	21
20	14	36	65	13	18	24
24	25	47	82	16	21	27
28	32	60	107	19	27	32
32	47	80	129	24	30	35
36	60	100	140	31	38	40
40	85	132	152	38	43	46
44	108	140	156	47	49	53
48	150	154	167	52	56	61

APPENDIX - B-VI

TABLE OF OBSERVATIONS

SUBSTRATE: Peptone
CONCENTRATION: 120 mg/lit.
COD: 138 mg/lit.

Time in Hours	BOD EXERTED IN mg/lit.			VIABLE CELL MASS IN mg/lit		
	R =1/48	R=1/24	R=1/12	R=1/48	R=1/24	R=1/12
4	-	2.0	4.0	3.0	5.1	11.0
8	1.0	5.0	8.5	4.0	6.0	12.6
12	2.5	7.5	13.5	4.2	7.2	14.0
16	5.0	11.0	17.5	5.3	7.6	16.0
20	8.0	16.0	26.0	6.1	9.8	16.8
24	8.5	18.0	34.0	7.8	11.0	19.8
28	12,5	23.5	38.0	9.0	13.2	21.4
32	16.0	27.5	47.0	10.8	15.0	23.0
36	19.5	33.0	56.0	12.6	19.1	26.0
40	22.5	40.0	65.0	14.4	22.0	27.2
44	27.5	44.0	72.5	16.8	25.8	31.0
48	35.0	54.0	76.5	19.8	29.0	32.4

APPENDIX - B-VII

TABLE OF OBSERVATIONS

SUBSTRATE: Sewage
COD : 182 mg/lit.

Time in Hours	BOD EXERTED IN mg/lit.			VIABLE CELL MASS IN mg/lit.		
	R =1/72	R=1/36	R=1/18	R=1/72	R=1/36	R=1/18
4	1	3	6	3.0	6.0	11.9
8	3	8	14	4.2	7.5	13.3
12	7	12	22	5.1	9.9	17.0
16	8	21	33	6.6	10.8	17.7
20	15	29	44	8.9	13.6	20.8
24	20	34	58	11.1	17.8	21.6
28	26	46	70	12.9	20.5	25.5
32	32	51	87	17.7	22.6	27.0
36	40	66	101	21.0	25.4	30.1
40	49	76	116	23.8	30.1	33.2
44	62	97	134	29.6	33.1	36.1
48	75	109	144	31.8	37.5	39.0

APPENDIX - C

TABLE FOR FLASK CONSTANTS

Flask Constant, $k = \frac{V_g (T_o/T) + V_f (\ )}{P_o}$

Where, V_g - volume of gas space in the vessel (including the connecting and manometer tubes down to the reference-mark)

T_o - Absolute Temperature (Kelvin) = 273°K

T - Absolute temperature of the thermostatic bath.

V_f - Volume of fluid in the vessel .

- Solubility of the evolved gas (CO_2) in the liquid in the vessel = 0.0261 (expressed as ul of gas at N.T.P. dissolved in 1 ul liquid when in equilibrium with a partial pressure of the gas equal to P_o)

P_o - Normal pressure in mm of manometric fluid, for Brodies solution, = 10000 mm.

*FLASK NUMBER	FLASK CONSTANT k in µl/mm
1	6.39
2	6.76
3	6.88
4 (Thermo barometer)	6.63

* Flasks of 125ml volume

V_f = 50ml

T = 25°C

APPENDIX - D-I

INTEGRATION OF THE DIFFERENTIAL EQUATIONS (10) AND (12)

1. BOD Equation:

$$-\frac{dS}{dt} = \frac{K_X K_{max} S X}{K_S + S} \qquad \text{where,}$$

$$\text{where,} \quad X = X_o + \frac{1}{K_X}(S_o - S)$$

Substituting the value of X in the above equation, and rearranging,

$$-\frac{dS\ (K_S + S)}{\left(X_o + \frac{1}{K_X}(S_o - S)\right) S} = K_X K_{max}\, dt \qquad (1)$$

Now,

$$\frac{K_S + S}{\left(X_o + \frac{1}{K_X}(S_o - S)\right) S} = \frac{A}{X_o + \frac{1}{K_X}(S_o - S)} + \frac{B}{S}$$

i.e. ~~A~~ i.e. $A(S) + B\left(X_o + \frac{1}{K_X}\overline{S_o - S}\right) = K_S + S$

At $S = S_o$, $A(S_o) + B(X_o) = K_S + S_o$ (2)

At $S = 0$, $B(X_o + (S_o/K_X)) = K_S$ (3)

$$B = \left(\frac{K_S}{X_o + (S_o/K_X)}\right)$$

Substituting this value in (2) and solving for A,

APPENDIX - D-I (Continued)

$$A = 1/S_o \quad (K_S + S_o) - \frac{K_S X_o}{X_o + (S_o/K_X)}$$

Substituting the values of A and B in (1), rearranging, and integrating both sides,

$$\int_{S_o}^{S} \left[1 + \frac{K_S}{K_X (X_o + \frac{1}{K_X} S_o)} \right] \frac{dS}{(X_o + \frac{1}{K_X} \overline{S_o - S})} + \int_{S_o}^{S} \frac{K_S}{(X_o + \frac{1}{K_X} S_o)} \frac{dS}{S}$$

$$= \int_o^t K_X K_{max} \, dt$$

i.e. $$- \frac{K_S}{X_o + \frac{1}{K_X} S_o} \left[- \log (X_o + (1/K_X) \overline{S_o - S}) + \log S \right]_{S_o}^{S} -$$

$$K_X \left[\log (X_o + (1/K_X) \overline{S_o - S} \right]_{S_o}^{S} = K_X K_{max} t$$

i.e. $$\frac{K_S}{X_o + (1/K_X) S_o} \left\{ \log \frac{X_o + (1/K_X) \overline{S_o - S}}{X_o} (S_o/S) \right\} +$$

$$K_X \log \frac{X_o + (1/K_X)(\overline{S_o - S})}{X_o} = K_X K_{max} t$$

i.e. $$\left[\frac{(X_o + (1/K_X) \overline{S_o - S}}{X_o} \right]^{K_X} = \frac{e^{K_X K_{max} t}}{\left\{ \frac{X_o + (1/K_X) \overline{S_o - S}}{X_o} (S_o/S) \right\}^{\frac{K_S}{X_o + (1/K_X) S_o}}}$$

i.e. $$(1 + (1/K_X X_o) \overline{S_o - S}) = \left\{ \frac{e^{K_X K_{max} t}}{\left[\frac{X_o + (1/K_X) \overline{S_o - S}}{X_o} (S_o/S) \right]^{\frac{K_S}{X_o + (1/K_X) S_o}}} \right\}^{1/K_X}$$